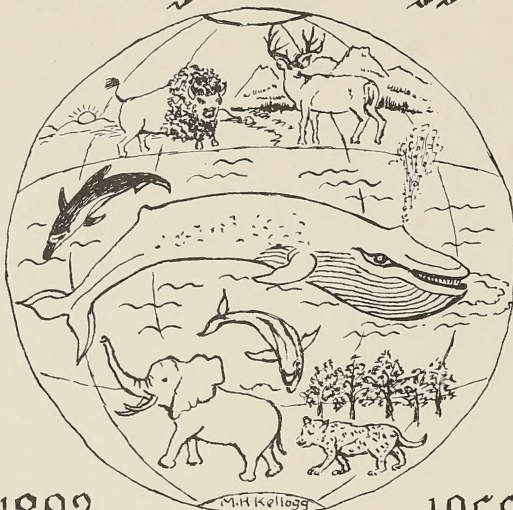


QH
431
C263X
Mamm.

CONTRIBUTIONS TO THE GENETICS
OF THE DOMESTIC RABBIT

W. E. CASTLE AND PAUL B. SAWIN

In Memory of
Remington Kellogg



1892

1969



Mammalogist
Paleontologist



0-6

Remington College

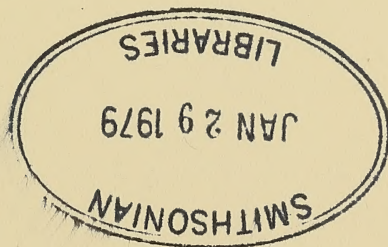
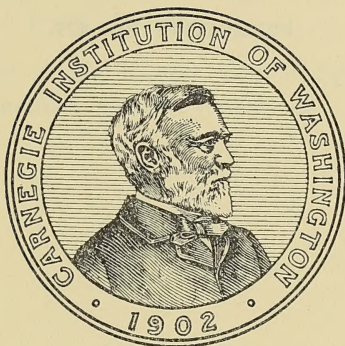
Division of Mammals

CONTRIBUTIONS TO THE GENETICS
OF THE DOMESTIC RABBIT

PH
431
C263X
Mamm.

CONTRIBUTIONS TO THE GENETICS OF THE DOMESTIC RABBIT

W. E. CASTLE AND PAUL B. SAWIN
///



PUBLISHED BY
CARNEGIE INSTITUTION OF WASHINGTON
1932

CARNEGIE INSTITUTION OF WASHINGTON

PUBLICATION No. 427

Hooper Printing Co., Boston
Standard Engraving Company, Washington.

CONTENTS

I English and Dutch Spotting and the Genetics of the Hotot Rabbit. (6 plates) By W. E. Castle.....	3-13
II Albino Allelomorphs of the Rabbit with Special Reference to Blue-eyed Chinchilla and its Variations. (6 plates) By Paul B. Sawin	15-50

I

ENGLISH AND DUTCH SPOTTING AND THE
GENETICS OF THE HOTOT RABBIT

By W. E. CASTLE
Bussey Institution, Harvard University

With six plates

CONTENTS

English	3
Dutch	4
Combined English and Dutch	6
The Hotot Rabbit	9
References	13

ENGLISH AND DUTCH SPOTTING AND THE GENETICS OF THE HOTOT RABBIT

In previous publications I have shown that English and Dutch patterns of white spotting are inherited as unifactorial Mendelian characters, the former behaving as a dominant, the latter as a recessive in crosses with unspotted (self) individuals. Each of the characters acts as an inhibitor of pigment production in particular regions of the coat and its action is stronger, that is larger white areas are produced, when its gene is homozygous (represented doubly) than when it is heterozygous (represented singly) in the zygote. But Dutch expresses itself very little in heterozygotes and so may properly be classed as recessive, although the difference between dominant and recessive characters in general is to be regarded as one of degree of expression rather than of kind. Both English and Dutch vary in expression through genetic agencies other than their heterozygous or homozygous state. In the case of English and to some extent also in the case of Dutch, such variation can be accounted for as due to modifying factors, which apart from the spotting gene itself would perhaps not produce a spotted coat.

ENGLISH

Plate 3 shows the ordinary range of variation of the English pattern. Figures 1 and 2 show its appearance in homozygotes, figures 3 to 6 in heterozygotes, as seen in "checkered giants." Special modifying genes are probably responsible for the more massed, fused and extended state of the typical spots, seen in figures 7 to 10. Other special modifiers are probably responsible for the pattern of very numerous and separate small spots found in the exhibition type of English seen in Plate 4, figure 1.

The English gene has its fullest expression in numerous distinct, colored spots on a white background in heterozygotes. The spots are carefully listed in the standards of fanciers. In homozygotes the size of these spots is diminished and many of them disappear altogether. Compare for example figure 1 with figure 2 of Plate 4, or figures 1 and 2 of Plate 3 with figures 3 to 6 of the same plate. The maximum of distinct, colored spots, which is desired by the fancier but is found only in heterozygotes, may be listed as follows.

A butterfly-shaped spot bestrides the nose. So characteristic is this marking in carefully bred strains of exhibition English or in Checkered Giants that such strains are frequently referred to as "butterflies." Not all individuals heterozygous for the English gene show this marking and it was not formerly insisted on as a feature of prize-winning animals. But absence of the butterfly on the nose is probably not due to a modification

of the English gene itself or to a different allelomorph of it, but rather to some modifying factor. Dutch can function as such a modifier, as I shall show, and very probably did so function in the less improved strains of English having a white nose. Inspection of Plate 1 will show that the first manifestation of a gene for Dutch is a white nose, a white fore-foot or a star in the forehead. A gamete carrying a low grade of Dutch (Plate 1, figs. 1-3) uniting with a gamete carrying the English gene will often produce an English individual with a white nose, as I have observed in crossing a small race of rabbits with Checkered Giants.

A circle of colored fur surrounds the eye, and there is a smaller distinct spot below it. The ears are colored but not uniformly. The pigmentation shows a tendency to break up into smaller spots, especially along its margins. A *spinal stripe* begins on the neck and continues with occasional interruptions out on to the dorsal surface of the tail.

Spots on either side of the spinal stripe are found chiefly on the middle of the back where they are largest and most numerous and extend farthest down the sides, but show a tendency to run together into a continuous dorsal blanket. Such fusion is not desired by the fancier who aims to secure by selection an open "herring-bone" pattern.

Lateral spots beginning on the shoulder continue backward and downward along the sides below the "herring-bone" and form the "chain." A *spot* is found near the middle of each leg (at morphological wrist and ankle). On the under side of the body, there is a colored spot *at each teat* and an imperfectly expressed *mid-ventral line*, counterpart of the mid-dorsal or spinal stripe. The *scrotum* or other *external genitalia* may be mottled.

A homozygous condition of the English gene causes many of these spots to disappear, or reduces the size of those which remain. This is better understood if we think of the English gene as causing the white areas rather than the colored spots. It produces the inhibition of pigment formation, of which the colored spots are mere residua, unaffected areas. Naturally, two doses of the English gene accentuate the inhibition and so diminish the unaffected areas.

Other inhibitors of pigmentation, genetically distinct from the English gene are the genes for Dutch marking and for Vienna White. When either of these is associated with the English gene in a zygote, they have a combined action to whiten the coat, that is the colored spots are diminished in number and size. Doubtless other genes also modify the expression of the English gene, as in the type of modification seen in figures 7 to 10 of Plate 3, though apart from the English gene they have no observable effect, but such genes have not as yet been individually identified.

DUTCH

The only known allelomorph of the English gene is non-English. The case of Dutch is more complicated. The entire range of variation of the Dutch pattern is shown in Plate 1. The prize-winning type of the fancier shown

in figure 8 is not ordinarily true breeding, but produces three types of Dutch marked animals in a 1:2:1 ratio. Two of these types (the less numerous ones) are true breeding (homozygous). I have called them Dark-Dutch and White-Dutch respectively (see Plate 2, figs. 2 and 3). Fancier's Dutch (Plate 2, fig. 1) is a heterozygote between these two and so not true breeding, in which it resembles the well-known blue Andalusian fowl. White-Dutch (Plate 1, figs. 9-17) differs from Dark-Dutch in the following respects: (1) White-Dutch usually has a wider white belt, (2) the area posterior to the belt is broken up into a number of small spots, and (3) the anterior surface of the iris is unpigmented, producing a blue or "wall" eye. I regard these two types as allelomorphs of the same gene and in support of this view have presented evidence that both show linkage with the same gene, *angora*. This, however, would not prove beyond question that they are allelomorphs, but only that their genes lie in the same chromosome, and Punnett and Nachtsheim question the allelomorph interpretation. But so many cases of multiple allelomorphism have now been established beyond question both for the rabbit and for other animals, that the allelomorph interpretation has much in its favor. At one time I described a third Dutch allelomorph "tan" Dutch combining some of the features of the other two, but it is possible that I was mistaken in this and that I was really dealing with a modified White-Dutch type, since it had wall eyes in some cases. Its pigmentation was in general more extensive than that of typical White-Dutch and it lacked the interrupted spotting posterior to the belt, but both of these differences may have been due to genetic modifiers. As the race is no longer extant, it is impossible to determine the matter by new experiments. The two types, White and Dark, are readily obtained at any time by inbreeding ordinary standard exhibition Dutch, and I am strongly of the opinion that they are allelomorphs. Occasionally, however, one finds a standard Dutch individual which is not a heterozygote of the two types but produces a uniform progeny when mated with a homozygous individual (White-Dutch, Dark-Dutch, or self). Hurst has described and figured such animals and I have recently obtained one from a fancier. They are in my experience, as in that of Hurst, rare as compared with the ordinary heterozygous type. They are probably modified Dark-Dutch, an explanation which I hope shortly to test by new experiments.

The genes for White-Dutch and for English, though producing very different phenotypes, are closely linked with each other, as I was able to show in 1926. They lie in the same linkage system as *angora* with which they show about 13 or 14 per cent of crossingover. In a cross of English with White-Dutch, F_1 animals are produced which are unmistakably English in pattern but have the typical English spots reduced in number and extent (see figs. 2 and 3, Plate 7, Castle 1926). The colored spots which persist are those of the eye and ear, usually also a spot or two representing remnants of the spinal stripe or of the chain of side spots. The eyes are full colored (brown) as in ordinary English individuals. The White-Dutch

pattern, recessive in the F_1 animals, has a whitening influence on the coat about equal to that of a second dose of the English gene, though it specifically eliminates all nose markings, which may persist, much reduced in extent, in homozygous English.

COMBINED ENGLISH AND DUTCH

A back-cross of such F_1 animals with homozygous White-Dutch produces as a rule equal numbers of (1) English individuals like F_1 in appearance and genetic constitution and (2) of homozygous White-Dutch. The latter have blue (wall) eyes, the former brown eyes. From such a back-cross was reported in 1926 only one individual in 730 which was not clearly of one class or the other. This individual was apparently English but different from the other English segregates in that (1) its colored spots were limited to the eye and a part only of each ear, (2) its eyes were blue, not brown, and (3) its coat was angora, not short. The reduced English spotting and the blue eye indicated a homozygous condition of the Dutch gene. But pigmentation at the base of the tail which is invariably present in even the whitest ordinary White-Dutch was in this animal entirely wanting, evidence that the English gene was present and inhibiting it. This interpretation was supported by the fact that the individual was long haired. For in the original cross an angora Dutch was mated with a short-haired English, and the back cross was made to angora Dutch. Hence the peculiar individual was apparently homozygous for the linked genes White-Dutch and angora, and at the same time heterozygous for English. The combination could have arisen only by the union of a gamete carrying Dutch and angora (furnished by the Dutch angora parent) with a similar gamete (furnished by the F_1 parent) to which had been added by a crossover the English gene. Hereafter in this paper this individual will be referred to as the crossover individual.

Concerning him I said in 1926 "Mated with homozygous White-Dutch females, he has produced four litters of young, part of which are ordinary White-Dutch and others very light English like himself, with no body spots whatever and very restricted head spots (eye and ear)." In the following pages I propose to give a fuller account of these and other progeny of the crossover individual.

The crossover male was mated with a considerable number of homozygous White-Dutch females including his own mother, and by them produced 148 young of which 71 resembled himself, showing the presence of an English gene in addition to a homozygous condition as regards White-Dutch. The other 77 young were ordinary White-Dutch individuals of grades ranging from 8 to 16 (table 1 and Plate 1). The 71 young which contained an English gene linked with one of the White-Dutch genes fall into four groups which will be indicated by the four grades (supplementary to those figured in Plate 3) 1, $\frac{1}{2}$, $\frac{1}{4}$ and 0. Grade 1 is used to indicate an animal which

TABLE 1—*Classification of the young sired by the original crossover English-Dutch male, 6559.*

Grade of English young										Grade of Dutch young									
Mothers		4	3	2	1	½	¼	0	Total	0	1-2	3-4	5-6	7-8	9-10	11-12	13-14	15-16	Total
White-Dutch (grades 12-17)		..	..	..	11	43	11	6	71	..	..	..	..	1	6	23	35	12	77
English Daughters (grades ¼-1)		..	..	..	13*	14	12	1	40	..	..	..	..	..	2	6	6	2	16
Self or Dark Dutch (grade 0-2).....		3	5	6	1	..	1?	..	16	6	7	5	..	..	..	..	..	..	18

*Eight of these had only one eye spot; one had a small back spot.

has eye-spots and also a small pigmented area on one or both ears; grade $\frac{1}{2}$ indicates that the individual has eye-spots only; grade $\frac{1}{4}$ signifies that the individual has a pigmented spot surrounding one eye only; and grade 0 indicates that the animal has no pigmented fur, though the eyes as in all the other groups are colored. The frequencies of these classes were 11, 43, 11 and 6 respectively (table 1). More than half the individuals (43) have both eyes surrounded with pigmented spots but are elsewhere white. This may be regarded as the modal phenotype of this genetic combination. The corresponding modal phenotype among the 77 White-Dutch young born in the same litters and presumably having a like combination of modifying factors lies within the range of grades 11 to 14, Plate 1 (58 individuals). The only genetic difference between the two types is that one contains an English gene in addition to the genetic complex of homozygous White-Dutch, whereas the other does not. When the English gene is absent, there is always a pigmented area of varying size at the base of the tail and on the rump; when the English gene is present this area is wanting, and the only pigmented fur is found in the eye spots or less often on the ears.

The crossover male was also mated with several of his crossover (English) daughters of grades $\frac{1}{4}$, $\frac{1}{2}$ or 1, producing 40 English-Dutch (crossover) young and 16 White-Dutch young, a fair approximation to the expected 3:1 ratio. The Dutch young were of grades 9 to 16, with a majority (12) in the middle groups (grades 11 to 14) as in the back-cross population, indicating a similar assortment of modifying factors. The English (crossover) young are of the same four types as in the back-cross group with frequencies of 13, 14, 12 and 1, respectively. The modal group is again that with a pair of eye-spots and no other pigmentation (grade $\frac{1}{2}$).

The crossover male was also mated with a few unspotted (self) females or such as were near self, being Dark-Dutch of grades 1 or 2 (table 1). In such matings he produced 18 young unspotted or low grade Dutch (grades 0-4), and 16 unmistakably English young of grades 1 to 4. All except one of the 16 English young had colored spots additional to eye spots, either ear spots, a partial back stripe, or side spots, or a combination of some or all of these. The one doubtful case is that of a yellow individual which died young. Although he was recorded as having only one pigmented eye spot, it is quite possible that inspection at a later age would have revealed pigmented areas on ears or body also. These matings show that with only one White-Dutch gene present, the one borne in the crossover gamete, a typical English pattern may be developed, whereas a homozygous state of White-Dutch associated with an English gene reduced the pigmentation to eye spots or at most to small additional ear spots. The same complex of factors which increases the pigmentation in the English group of young to grade 4 English, apparently increases the pigmentation in the Dutch group to grade 0 (self), *i.e.*, completely obliterates Dutch markings.

Certain of the crossover young sired by the original crossover male were mated inter se or with typical White-Dutch animals. The young produced by these matings are classified in table 2. The young derived from matings

with White-Dutch fall into two classes as did the young produced in similar matings by the original crossover male. The crossover (English) young number 55 distributed in the four grades 1, $\frac{1}{2}$, $\frac{1}{4}$, and 0, with frequencies of 2, 21, 16, and 16, respectively. The modal group is again grade $\frac{1}{2}$ with eye spots only. The Dutch young range in grade from 10 to 15, but number 27 only. The reason why the Dutch young are in this group of matings less numerous than 50 per cent, as in the similar matings of the original crossover male, is that certain of the parents were homozygous for the crossover combination, English-Dutch, and so produced only crossover young.

TABLE 2—*Later descendants of the crossover English-Dutch male, 6559.*

Parents	Grade of English young					Dutch young (grades 10-15)
	1	$\frac{1}{2}$	$\frac{1}{4}$	0	Total	
English (0- $\frac{1}{2}$) x White-Dutch	2	21	16	16	55	27
English (0-1) bred inter se	8	77	75	85*	245	28

*One with a small tail spot.

In the matings inter se of crossover (English-Dutch) individuals of grades 0-1 (table 2), there were also produced less than 25 per cent of Dutch young for the reason just stated, that some individuals were crossover homozygotes. The English-Dutch young produced by such matings numbered 245, with a great majority of them having only eye-spots or no colored fur at all. One individual which had no spots on the head had a small spot on the tail. Most of them had blue (wall) eyes like the original crossover male and White-Dutch individuals in general. No particular record was made of this feature until a comparative study came to be made of the Hotot rabbit. Then it was found that an occasional individual among them had brown eyes, or eyes partly brown (mosaic). This may be explained as due to the evenly balanced but opposite tendencies of the linked genes English and Dutch. Typical English individuals, whether homozygous or heterozygous, are brown-eyed; White-Dutch homozygotes are blue-eyed. In crossover individuals the homozygous state of the Dutch gene usually produces blue eyes, but the tendency of the English gene to produce a brown eye may counteract the influence of the Dutch gene partially or wholly, so as to produce mosaic or wholly brown eyes.

It may be observed in table 2 that the modal class of crossover young produced in the inter se matings is the grade 0 group. Parents of that group were given preference in the matings made in the later generations. This perhaps explains why that group came to predominate, through the segregation of a particular set of modifying factors.

THE HOTOT RABBIT

Blanc de Hôtot¹, Plate 6, figure 1, is the name of a breed of large-sized white rabbits kept in France and distinguished by a peculiar and striking color pattern. The animals are entirely white except for a circle of colored

¹ Hereafter in this paper for simplicity Hotot.

fur surrounding each eye. The eyes themselves are colored. In the two animals which I have seen the eyes were brown, as in ordinary self-colored rabbits. Whether there is any variation in the eye color within the breed, I have had no opportunity to observe.

When I first learned of the existence of this breed of rabbits I was struck by its close resemblance to the modal class of my English-Dutch crossover race, having eye spots only, and I sought an opportunity to compare the two races genetically. Only recently have I been able to do so. Through the kindness of Dr. Wade Wright of the Metropolitan Life Insurance Co. of New York who imported some Hotot rabbits from France and gave them to Dr. Wade Brown of The Rockefeller Institute, I received two male individuals in 1930. My hearty thanks are due to both these gentlemen for generously providing me with experimental material which I had sought in vain elsewhere in America. From one of the animals which I thus obtained (Plate 6, fig. 1) numerous offspring have been secured in crosses presently to be described and these throw light on the genetic constitution of the breed. When Hotot is crossed with self-colored rabbits, offspring are produced which have the characteristic markings of English rabbits, colored areas additional to the eye spots being produced regularly on the ears, and in a spinal stripe, less often also on the sides, tail and nose (see Plate 6, fig. 2). This result supports Nachtsheim's opinion that Hotot is in some way derived from the English mutation.

When the Hotot male is mated to his English marked F_1 daughters from the cross with self does, two classes of young are produced in equal numbers. One class is typical Hotot in character with a spot surrounding one or both eyes but with no other spots. The other class consists of English marked animals like their F_1 mothers and presumably like them heterozygous. These bear, in addition to eye spots, ear-spots and usually also back spots, side spots or nose spots, or a combination of all of them. It is evident from this result that the Hotot character segregates as a unit, but varies in expression as does the English-Dutch unit of two closely linked genes, since the eye spot may be present on one side only, or on neither side (grade 0).

An F_2 generation from Hotot crossed with self consists of three types in approximately a 1:2:1 ratio. These are respectively, selfs, English of about grade 2 (like the F_1 parents) and extracted Hotot. The first and last mentioned groups are obviously homozygotes, the middle group heterozygous.

In three such F_2 litters there were recorded 5 self individuals, 9 English of grades $1\frac{1}{2}$ or 2, that is with spots additional to eye spots, and 8 with eye spots or no spots at all.

The last group included one individual with two eye spots, 2 individuals with one eye spot each, and 5 individuals with no eye spots (grade 0). It is important to note that certain of the extracted homozygous Hotot individuals had mosaic eyes similar to those found in the English-Dutch race.

My own "crossover" race, which resembles Hotot, also produces English marked young in crosses with self (see Plate 5, fig. 3). Its genetic constitution is known to be a synthesis of English with the particular type of

Dutch which I have designated White-Dutch, and for this reason I have suggested elsewhere (Castle 1930) that Hotot may have resulted from a similar synthesis. This suggestion is strongly supported by the experimental evidence which I shall presently summarize.

The chief difference between the Hotot male which was used in my experiments and my synthetic English-Dutch race consists in the eye color. The Hotot male has a brown eye, my English-Dutch rabbits usually have a blue eye (Plate 4, fig. 3), but I find among them, on making a careful inspection of my stock, one which has brown eyes (Plate 5, fig. 2), one which has the right eye chiefly brown (mosaic) and the left eye blue. Compare Plate 6, figures 3 and 4. It is accordingly possible for the English-Dutch phenotype to be brown-eyed partly or wholly, though in my experience it is usually blue. Brown eye, with the anterior wall of the iris pigmented, is characteristic of English; blue eye with the anterior wall of the iris unpigmented is characteristic of White-Dutch. In Hotot and my English-Dutch race the two tendencies seem to be pretty evenly balanced, with brown eye predominating in the former, blue eye in the latter.

In crosses of the two races with each other, the balanced state is expressed still more strongly. The Hotot male (a homozygote as regards Hotot pattern) having brown eyes has been mated both with English-Dutch having blue eyes and with pure White-Dutch having blue eyes. The results are similar in both cases. Combining them, we have in a total of 27 young, 12 which are blue-eyed, 6 which have one eye blue, the other mosaic, 3 which have both eyes mosaic, and 3 which have one eye blue, the other brown. Three young died before their eye character had been determined.

As to eye-spots in this group of young, 14 individuals have the spot of colored fur round each eye, 10 have one eye spot only, 3 have none. No other spots than eye-spots are developed in any of the young. In other words the phenotype common to both parent races is present in the great majority of their offspring, and none deviate from it in the sense of adding spots characteristic of English unassociated with Dutch (ear spots), or of Dutch unassociated with English (rump or tail spots).

The evidence that Hotot and my synthetic English-Dutch race are of like genetic constitution may then be summarized thus:

1. The two are similar in appearance (phenotypically).
2. They reproduce the common phenotype when mated with each other.
3. Both produce similarly marked English young, which are brown eyed, when mated with an unspotted (self) race.
4. Both show simple segregation of the typical condition when a back-cross is made of the English marked F_1 animals with the parent type, and the type segregates vary in a similar way.
5. Both produce similarly marked Hotot type young when mated with White-Dutch. These young have eye spots only, or rarely no spots at all in the fur, and their eyes are either blue or mosaic, or at most they have only one eye brown.

6. An F_2 from Hotot mated with self produces extracted Hotot animals, some with and others without eye spots, also some with mosaic, others with uniformly pigmented eyes, a parallel variation to that found in the English-Dutch race.

There is accordingly every reason to think that the Hotot rabbit arose, as the synthetic English-Dutch race is known to have arisen, by a crossover between the closely linked genes English and White-Dutch in an individual heterozygous for both.

POSTSCRIPT

It might be well to consider the bearing of these observations upon the conception of "step allelomorphism" presented by Agol ('31). He holds that genes are not discontinuous units composing chromosomes, but that "the entire chromosome is continuous" and "one gene passes directly into another," the component genes or portions of the chromosome being themselves composite. Complete allelomorphism is found only when exactly corresponding parts of a pair of chromosomes are considered, these parts having equivalent somatic effects. In the example which he elaborates, a gene scute is considered to be completely allelomorphic to another gene scute only when it affects exactly the same quota of bristles on the body of *Drosophila*. But if it affects only a part of these bristles, it is only partially allelomorphic and must be given a different designation as scute¹, scute², etc. Multiple allelomorphs on this view are portions of homologous chromosomes coming opposite each other in synapsis *in part* but overlapping so that one includes portions not included in the other.

The concept seems to be based on a comparison of somatic effects, it being assumed that identical somatic effects are due to identical genetic agencies. This is demonstrably not true. White fur in rabbits may result from a variety of genetic agencies, lying in different linkage systems (chromosomes), and so not allelomorphic on any conception of allelomorphism yet formulated.

Genes of the rabbit which produce white fur are (1) the gene for albinism, *c*, which is located in the same chromosome with genes *b* (brown) and *y* (yellow fat), (2) the genes for White Dutch and for English which are located in the same chromosome with *l* (angora), (3) the gene for Vienna White which lies in a third chromosome. On Agol's conception, English and Dutch might be considered step allelomorphs, since they have physiological effects which are similar in certain parts of the coat, and are demonstrably located in the same chromosome at loci not far apart.

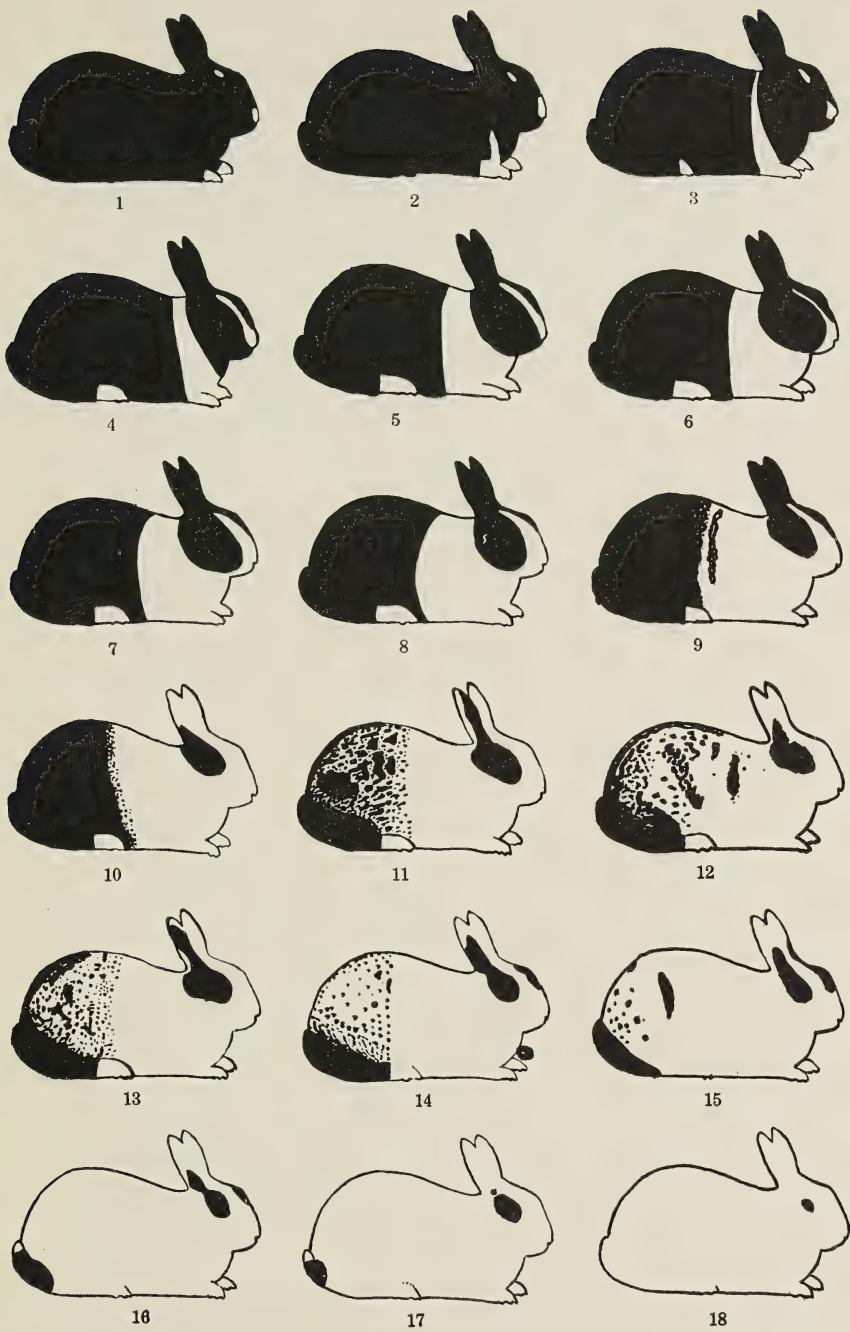
But Vienna White has local somatic effects (especially in heterozygotes) which closely resemble those of White Dutch. The resemblance is much closer than that between English and Dutch. Yet the gene for Vienna White is not borne in the same chromosome as White Dutch, English and Angora, and so can not be considered an allelomorph of White Dutch on the conception of allelomorphism formulated either by Agol or by Morgan.

Our present knowledge agrees better with the conception of a gene as an organic compound of definite and stable constitution found ordinarily only at a particular locus of a particular chromosome. Allelomorphs of the same gene differing in their somatic effects doubtless differ in their constitution, perhaps analogous with the differences in chemical isomeres.

It is quite conceivable that by translocation, true allelomorphs might come to lie in non-homologous chromosomes, in which case they might no longer be recognizable as allelomorphs, but would function as duplicate genes. Such cases are probably rare and have been demonstrated thus far only in the case of plants in which polyploidy is common.

REFERENCES

- AGOL, I. J. 1931. Step allelomorphism in *Drosophila melanogaster*. *Genetics*, vol. 16, pp. 254-266.
- CASTLE, W. E. 1926. Studies of color inheritance and of linkage in rabbits. *Carnegie Inst. Wash. Pub. No. 337*, pp. 1-47, 8 plates.
- . 1930. The genetics of domestic rabbits. *Harvard Univ. Press, Cambridge, Mass.*, 31 pp., 39 figs.
- HURST, C. C. 1925. 1913. Breeding experiments with Dutch rabbits. *In Experiments in Genetics*, pp. 460-461. *Cambridge (Eng.) Univ. Press.*
- NACHTSHEIM, HANS. 1929. Die Entstehung der Kaninchenrassen im Lichte ihrer Genetik. *Zeit. f. Tierzüchtung und Zuchtungsbiobiol.*, 14, pp. 53-109, 10 text-figs. and 3 plates.



Grading scale for classification of Dutch rabbits. Grade 8 is type desired by fanciers. Grades 9 to 17 are types homozygous for the White-Dutch gene, which differ among themselves through the action of modifying factors. Grade 18 is the Hotot, or combined English-White Dutch type. Compare Plates 5 and 6.

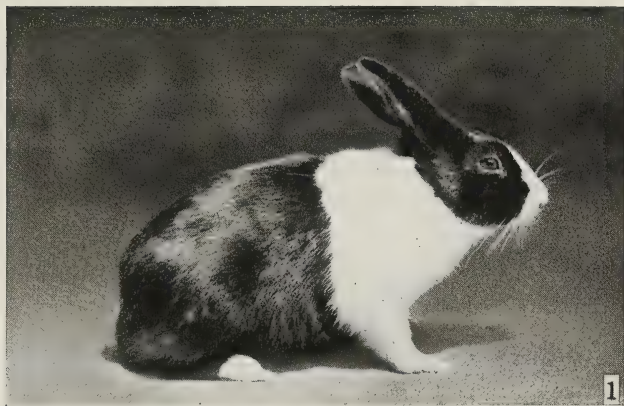
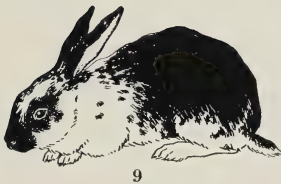


FIG. 1—Standard exhibition Dutch rabbit.

FIG. 2—White Dutch of grade 15 (Plate 1). Note unpigmented iris (wall eye).

FIG. 3—Homozygous Dark Dutch of grade 5 or 6.



Grading scale for classification of English rabbits. Grades 1 and 2 as seen in homozygous "checkered giants," grades 3 to 6 as seen in heterozygous "checkered giants." Grades 7 to 10 show dark modifications of English due to special genes. After drawings by Dr. C. E. Keeler.

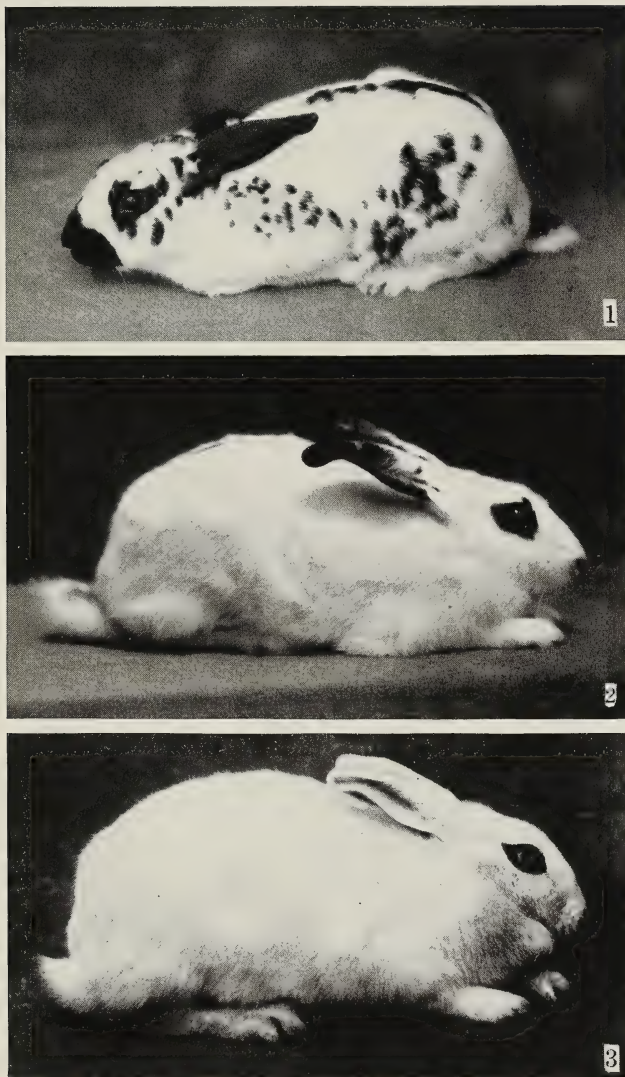


FIG. 1—Type of exhibition English desired by the fancier, heterozygous for the English gene.

FIG. 2—Homozygous English with spots restricted to eyes, ears and spinal stripe.

FIG. 3—A typical crossover English-Dutch rabbit, homozygous for White Dutch, heterozygous for English, wall-eyed and with a spot of non-agouti black fur around each eye.

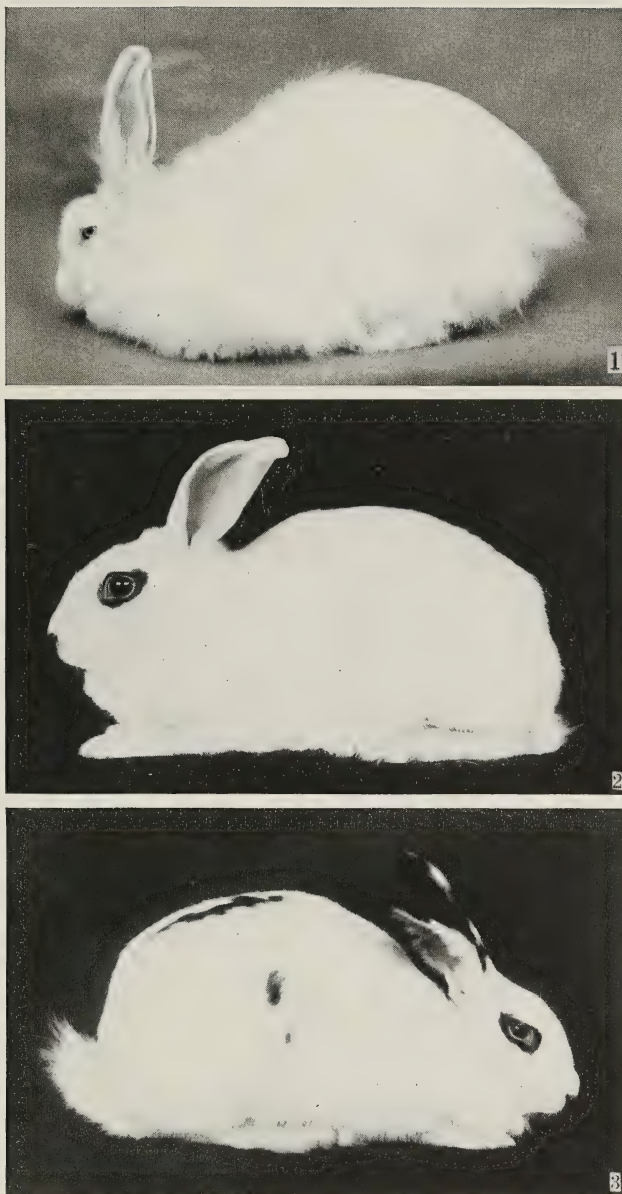


FIG. 1—English-Dutch angora, homozygous for all three linked genes and wall-eyed.

FIG. 2—An English-Dutch rabbit of grade $\frac{1}{2}$ (with eye-spots only). It is homozygous for White Dutch and heterozygous for English, but its eyes are brown and so in all respects it resembles Hotot rabbits.

FIG. 3—F₁ rabbit from a cross of such an English-Dutch animal as is shown in Plate 4, figure 3, with a self (unspotted) mate. Its eye spot is of non-agouti black fur. Compare with Plate 6, figure 2.

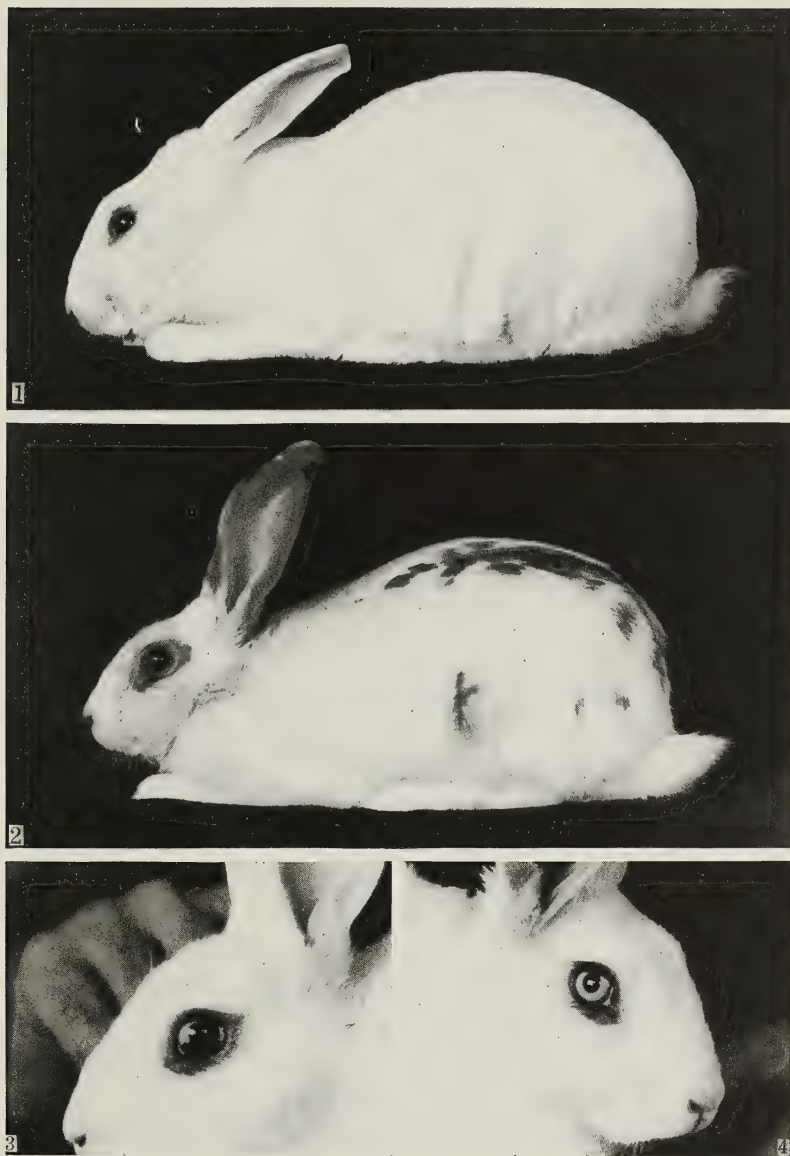


FIG. 1—The original Hotot male, with brown eyes and gray fur in its eye-spots.

FIG. 2—F₁ gray English rabbit produced by crossing the Hotot male shown in figure 1 with a blue self female. Compare with Plate 5, figure 3.

FIGS. 3 and 4—Left side and right side respectively of head of a rabbit with gray eye spots (English grade $\frac{1}{2}$) whose sire was the Hotot male figure 1, and whose mother was an English-Dutch (compare Plate 4, fig. 3). The left eye is mosaic, the right blue. In the coat, eye spots only are pigmented.

II

ALBINO ALLELOMORPHS OF THE RABBIT WITH
SPECIAL REFERENCE TO BLUE-EYED
CHINCHILLA AND ITS VARIATIONS

By PAUL B. SAWIN

With six plates

CONTENTS

Introduction	17
Albino series of the rabbit	18
Full color	18
The albino allelomorphs	19
The chinchilla allelomorphs	19
Materials and methods.....	20
Brown-eyed dark chinchilla ($ch^3 ch^1$)	21
Influence upon coat color and eye color of albinism in heterozygous combinations	23
Test for linkage between blue eye and dark chinchilla	24
Variation in eye color	26
Heterochromia iridis in dark chinchillas	31
Histological study of grades of eye color	33
Full color	34
Blue-eyed dark chinchilla	34
Pale chinchilla	35
Rusty back	35
Relation of rusty back to eye color.....	40
Chinchilla combinations	40
General discussion of eye color	43
Wide ticking	45
Summary	47
Acknowledgments	48
Literature cited	49

ALBINO ALLELOMORPHS OF THE RABBIT WITH SPECIAL REFERENCE TO BLUE-EYED CHINCHILLA AND ITS VARIATIONS¹

INTRODUCTION

Albinism in the rabbit, as in the mouse, rat and guinea-pig, was one of the first Mendelian characters to be studied. Castle showed in 1905 that the pure white type of albino rabbit is allelomorphic to the Himalayan type with pigmented extremities, and Sturtevant (1913) used this case as an argument in favor of the view that a unit character can have more than two allelomorphic forms (dominant and recessive), since full color, complete albinism and Himalayan albinism form a series of triple allelomorphs. Wright (1915, 1916) in his studies of the guinea-pig established the existence of four different albino allelomorphs, forming a graded series in the amount of pigment produced. His data show that these grades are entirely discontinuous and completely allelomorphic. Later, after the discovery of a fifth allelomorph (1923) and a very extensive study of the entire series (1925), he has suggested "that the members of the albino series in mammals determine the rate of some one process fundamental to all pigmentation and that the irregularities in the order of effect on different kinds of pigment, or in different regions of the body, are due to subsequent physiological processes with which the albino series has nothing to do." In general the higher grades of pigmentation are dominant over lower grades in rabbits as in mice and guinea-pigs, but the dominance is often incomplete, particularly in the case of the lower grades of pigmentation.

In 1921 Castle showed that the chinchilla rabbit, like the silver agouti guinea-pig, is an allelomorph of albinism and full color, and in 1926 he established the fact that two different chinchilla allelomorphs can be recognized, making five albino allelomorphs in all. What was considered a sixth was described by Kosswig (1927) and Nachtsheim (1929) as occurring in the "Marder" rabbit. Kosswig was also able to show that the Marder varies in depth of pigmentation with the temperature, as had previously been shown by Schultz and others to be true of the Himalayan rabbit.

The various standards for chinchilla rabbits in use by American rabbit fanciers have recognized three distinct types of eye color in the chinchilla as brown, gray and blue. All three types were found to occur in the progeny of pure bred brown-eyed dark chinchillas in possession of the writer in 1927. An attempt to analyze this variation in eye color and its relation to the members of the albino series has led to a somewhat extended study of the

¹ Based on a thesis submitted to the Faculty of the Bussey Institution, Harvard University, in partial fulfilment of the requirements for the degree of Doctor of Science.

entire series. It is of special interest in throwing further light on the nature of the albino mutants.

Proof is presented in this paper of the existence of at least three different chinchilla allelomorphs, making a total of six allelomorphs in the albino series of the rabbit. These, in order of decreasing intensity of coat pigmentation are (1) full color; (2) dark chinchilla, which will be designated chinchilla No. 3; (3) light chinchilla or chinchilla No. 2; (4) pale chinchilla or chinchilla No. 1; (5) Himalayan albinism; and (6) pure white albinism. Dark chinchilla (ch^3)¹ seems to correspond fairly well with Castle's "dark" chinchilla, although as defined by him this might have included light chinchilla (ch^2) also. Nachtsheim's "Marder" almost certainly is identical with Castle's "pale chinchilla" which is the pale chinchilla (ch^1) of the present paper.

Blue eye is characteristic of the darkest chinchilla allelomorph, which will be referred to here as dark or No. 3 chinchilla. Gray eye and one type of brown (pseudo-brown) are modifications of the blue type effected by genes inherited independently of the albino series.

A certain amount of yellow pigment described by Castle as sometimes occurring in the chinchilla coat and known to fanciers as "rusty back" appears to be inherited independently of albinism.

A new genetic factor affecting the width of the agouti band of the secondary guard hairs and wool hairs of the chinchilla is also here described for the first time.

ALBINO SERIES OF THE RABBIT

FULL COLOR

The gene responsible for the development of full color in the coat is dominant over all of its allelomorphs.² Its combination with any one member lower in the series is indistinguishable from a combination with any other member of the series or from homozygous full color. Animals bearing the gene for full color are capable of developing both black and yellow to their fullest extent. Black, however, may be modified in different ways by other genes such as chocolate (b), dilution (d), and extension (E , E^p , e). Yellow may vary from intense rufous red to fawn and cream, influenced by such genes as dilution (d), and certain modifying genes described by Pap (1926) which, however, have never been satisfactorily analyzed. The eye in a rabbit of full color is brown, but is influenced by dilution and by chocolate. Full color is not known to be influenced by environmental agencies.

¹ Following the system of symbols established by Bateson and elaborated by Castle, full color is designated C , chinchilla ch , Himalayan ch^H and true albinism c . The three chinchilla types would then be ch^1 , ch^2 and ch^3 . For simplicity I shall in this paper use the shorter forms ch^1 , ch^2 and ch^3 .

² Three cases have been cited by Snell (1931) of incomplete dominance of full color in the house mouse, but so far as I know there are no authentic similar cases in the rabbit.

THE ALBINO ALLELOMORPHS

There occur as mutations from the full color of the wild type of rabbit two forms of albinism, the Himalayan and the true or complete albino. Their inheritance was first described by Castle (1906) as that of Mendelian recessives. This result was confirmed by Hurst (1906) and Punnett (1912). From the true albino, melanic pigment is entirely absent. The skin and iris are white and the pupil of the eye is pink, due to the reflection through it of the red blood in the tissues behind it. Pigment in the skin of the Himalayan albino is entirely absent at birth but a thermolabile pigment develops under normal environmental conditions after birth, producing the well-known markings of the extremities, nose, ears, feet and tail. The numerous studies of this phenomenon made by Schultz (1918, 1922, 1927 and 1930) and of Lenz (1923) and Iljin (1926) show that the development of pigment at the extremities is the result of the lower temperature of these parts and may be produced in other regions also by temperature control. The pupil of the eye is pink in the Himalayan as in the true albino, and the iris is of a milky gray color but it is capable of developing pigment under certain conditions as shown by Schultz (1927, 1929) by means of tissue cultures and transplants.

THE CHINCHILLA ALLELOMORPHS

The origin of the chinchilla allelomorphs is obscure. The chinchilla rabbit first came to the notice of fanciers in France during the world war. In 1920 chinchillas then imported into this country were producing poorly colored individuals which in the non-agouti form have since come to be well known and prized as Sables. Castle (1921) obtained a pair of chinchillas from England and in 1926 described, as occurring among their progeny, two forms having degrees of pigment intermediate between full color and Himalayan albinism. "Dark" chinchilla he described as producing intense black pigmentation with a certain amount of yellow in agouti individuals, restricted to the yellow ticking of the guard hairs of the back. Nachtsheim (1929), who like Castle has produced the non-agouti form of chinchilla, finds it difficult to distinguish from the full-colored non-agouti (black). The eye color of chinchilla was described by Castle as gray with a red glow by reflected light. Nachtsheim described the eye color of German dark chinchillas as brown, although blue or gray eyes are now known in chinchilla rabbits in Germany as indicated by Schultz (1930). American fanciers desire the brown eye, but have been greatly troubled with the segregation of blue and gray eyes from pure bred, brown-eyed chinchilla stocks.

"Pale" chinchilla, as described by Castle, lacks yellow pigment completely and has a reduced amount of black, making the individual appear much paler. The non-agouti form was designated by him "sepia." Fanciers now call it sable. The descriptions and figures given by Castle (1926) for "pale" and those given by Kosswig (1927) and Nachtsheim (1929) for

"Marder" suggest strongly the identity of the two. It seems probable that Marder is actually a non-agouti combination of the allelomorph which Castle described as pale (see Plate 3 of Castle, figs. 4 and 8 of Kosswig, and Plate 2 of Nachtsheim).

The pigment of the Marder, like that of the Himalayan albino, has been shown by Kosswig (1927) and Schultz (1930) to be subject to the influence of temperature. The optimum temperature for pigment formation is found by Schultz to be higher in the Marder than in the Himalayan, and the temperature gradient from one part of the body to another appears to be responsible for the variation in body color, the sides being characteristically lighter in the Marder heterozygous for complete albinism, while the extremities, as in the Himalayan, are darker.

The existence of an intermediate form distinct from pale, and also from dark chinchilla, is indicated in the "dunkel sepia" of Kosswig (1927) but experimental evidence that it represents a distinct chinchilla allelomorph was not presented. It lacks yellow pigment and shows a slight reduction of black as compared with dark chinchilla. I shall designate it light chinchilla (ch^2). The eye color of "dunkel sepia" and "Marder" has been described by Kosswig as dark brown with a red glow by reflected light.

MATERIALS AND METHODS

This study was originally begun with what were reported to be pure-bred, brown-eyed dark chinchillas, but they proved to be, like the chinchillas of Castle, heterozygotes of two chinchilla allelomorphs which I have designated pale chinchilla (ch^1) and dark chinchilla (ch^3) (see figs. 2 and 1). The former I believe to be identical with Castle's (1926) "pale" and the German "Marder." A direct comparison with the latter has been made possible by the use of a Marder buck, placed at my disposal by Dr. Castle. It was generously sent to him at his request by Dr. Nachtsheim, and was brought to this country by Dr. Walter Landauer in September 1930. This male differs in no way from my non-agouti pale chinchillas, which are heterozygous for albinism (see figs. 7 and 8). When mated to the same albino stock as has been used in my other experiments, he has produced five offspring like himself and four albinos.

Dark chinchilla is found in blue-eyed dark coated animals, corresponding in a general way with the gray-eyed dark chinchilla of Castle (1926) and the blue-eyed chinchilla of Schultz (1930). Various matings involving these two types as shown in table 2 had been made previous to my taking up study at the Bussey Institution in 1929, and it was considered probable that the two types were identical with Castle's dark and pale types. On examination of my animals by Dr. Castle this assumption was not verified with respect to the eye color of dark chinchilla. It was also found that the chinchilla animals, then in the Bussey stock and which seemed to be breeding true, differed from my dark chinchillas (1) in having a slightly

less intensity of black pigment in the coat, (2) in the total absence of yellow from the coat, and (3) in a slight tendency to thermolabile pigment production (*i.e.*, responsiveness to temperature differences), (4) in having a brown eye and (5) in being incompletely dominant over albinism. This Bussey race was not the one originally studied by Castle but had been obtained from Dr. Nachtsheim's laboratory in some animals heterozygous for the rex gene. Their non-agouti forms correspond closely with the "Dunkel sepia" form of Kosswig. It seems probable therefore that these two are identical and they will be designated in this paper *light chinchilla*. In table 1 are shown what I regard as the probable equivalency of the terms used in classifying chinchilla rabbits by Castle, Nachtsheim and Kosswig, and the present paper.

TABLE 1—*Corresponding types of chinchilla in classifications of Castle, Nachtsheim and Kosswig and the present paper.*

Castle	Dark chinchilla, eyes gray		Pale chinchilla
Nachtsheim and Kosswig	Dark chinchilla, eyes brown	Pale or "Dunkel sepia," eyes brown (non-agouti form)	Marder or Sable, eyes brown (non-agouti form)
Sawin	Dark chinchilla (ch^3), eyes blue, gray, or pseudo-brown (non-agouti form, intense black)	Light chinchilla (ch^2), eyes brown (non-agouti form, dull black)	Pale chinchilla (ch^1), eyes brown (non-agouti form, sable)

Records have been kept of all offspring produced, as to intensity of black pigmentation, rustiness of the back and eye color. Intensity of black has been noted at birth, all three characters have been noted at six weeks of age and records have been made at several other periods prior to maturity, especially in the earlier matings. Comparisons of coat color and rustiness of the back have been made by the use of a prepared series of skins supplemented by examination of living animals. An attempt was early made to grade the sepias and yellows by the use of Ridgway's color standards, but this method did not prove as satisfactory as the use of prepared skins, and so was discontinued. The method of comparing eye colors will be discussed later.

BROWN-EYED DARK CHINCHILLA (CH^3CH^1)

At the beginning of this study several matings inter se were made of the supposedly pure bred, brown-eyed chinchillas (mating 1, table 2). It soon became evident that these rabbits, like those of Castle (1926) were heterozygous for two types of coat color but, unlike his, they were also heterozygous for eye color. The eyes of the parents appeared to differ in no way from those of full-colored brown-eyed rabbits available for comparison.

From this mating 152 offspring were produced, of which 123 were raised to an age of 6 to 8 weeks or beyond. The latter consisted of 32 blue-eyed dark chinchillas, 58 brown-eyed dark chinchillas like their parents, and 33 brown-eyed brown-sided chinchillas later designated pale chinchillas. These numbers are a close approximation to a 1:2:1 ratio.

When blue-eyed dark chinchillas derived from mating 1 were mated inter se (mating 2, table 2) 25 offspring were produced, all blue-eyed dark

TABLE 2—*Classification of young produced by matings involving dark vs. pale chinchilla and blue vs. brown eyes. Numbers in parenthesis show theoretical expectations.*

Mating No.	Parents	Blue-eyed dark chin.	Brown-eyed dark chin.	Brown-eyed pale chin.	Totals
1	Brown-eyed dark chin.	32 (30.7)	58 (61.5)	33 (30.7)	123
2	Blue-eyed dark chin.	25 (25)	..	..	25
3	Brown-eyed pale chin.	..	..	20 (20)	20
4	Brown-eyed dark x brown-eyed pale	..	34 (36.5)	39 (36.5)	73
5	Brown-eyed dark x blue-eyed dark	48 (54.5)	61 (54.5)	..	109
6	Blue-eyed dark x brown-eyed pale	..	17 (17)	..	17
					367

chinchillas like their parents. When the brown-eyed pale chinchilla offspring of mating 1 were mated together (mating 3, table 2) only brown-eyed pale chinchillas were produced, 20 in number. This result indicates that the blue-eyed dark chinchilla and the brown-eyed brown-sided or pale chinchilla are both homozygous and due to different allelomorphs of the same gene. If so, brown-eyed dark chinchillas should be heterozygotes of the two types. This interpretation is supported by the results of backcross matings. Brown-eyed dark chinchillas mated with brown-eyed pale chinchillas (mating 4, table 2), both selected from the offspring of mating 1, produced 73 offspring of which 34 were brown-eyed dark chinchillas and 39 were brown-eyed pale chinchillas. The departure from equality of the two classes is only 2.5 which is less than the probable error, 2.8, and so is not statistically significant. This accordingly may be regarded as a 1:1 ratio.

In mating 5 a backcross was made of brown-eyed dark chinchillas with blue-eyed dark chinchillas and 109 offspring were produced, all dark chinchillas, but 48 were blue-eyed and 61 were brown-eyed, a deviation from equality of 6.5. The probable error for this number of offspring is 3.23, which is just about half the size of the observed deviation, which accordingly is probably not significant but due to random sampling. If dark coat (a dominant character) and blue eye (a recessive) were independently inherited, we should expect the double recessive recombination, pale coat with

blue eyes, to occur in one in sixteen of the F_2 individuals, but no such combination occurs in any of the matings recorded in table 2.

Dominance of dark coat and of brown-eye is shown particularly well in mating 6, in which the two true breeding types are crossed (dark chinchilla with blue eyes x pale chinchilla with brown eyes). The 17 offspring produced are all dark coated and have brown eyes.

If we turn again to mating 1 and assume that blue eye is due either to a gene independent of dark coat or to one loosely linked with it, we expect a recombination class to occur in which blue eye will be associated with pale coat, but no such class has been observed. But if we assume that dark coat and blue eye are effects of one and the same gene (or of two genes closely linked) and that brown eye and pale coat are likewise manifestations of a single gene (or two closely linked), then only the three observed classes of individuals are to be expected in the observed ratio of 1:2:1. The expected ratio in a population of this size is 30.75 blue-eyed dark chinchillas: 61.50 brown-eyed dark chinchillas and 30.75 brown-eyed pale chinchillas. The observed numbers deviate from these expectations by 1.3 ± 3.24 , 3.50 ± 3.74 and 2.3 ± 3.24 , respectively. The probable error in the second class is computed on the expectation that this class will comprise half the total population, and in the case of the other two classes that each will comprise one-fourth of the total. The deviation in each class is so slight as to be less than the probable error and thus to conform exactly with a 1:2:1 ratio. Furthermore if the linkage were not quite complete, that is to say if a small amount of crossing over were occurring, the second or double dominant class should be larger than the expectation calculated and the other two classes should be smaller. The observed deviations are of an exactly contrary sort.

These data gathered in part for other purposes indicated a close association of blue eye with dark coat and suggested making additional tests.

INFLUENCE UPON COAT COLOR AND EYE COLOR OF ALBINISM IN HETEROZYGOUS COMBINATIONS

If blue eye is one of the manifold effects of a dark chinchilla allelomorph and is not transmitted even potentially by albinos, then when this gene is combined with albinism only blue-eyed dark chinchillas should result. But if brown-eyed individuals are produced in such matings, the brown eye must have been derived from the albino parent.

The first mating indicating an inability of the albino to transmit brown eye was made between a brown-eyed dark chinchilla (known from pedigree to be heterozygous for pale chinchilla) and an albino of the breed known as New Zealand White. This breed, except as regards the color factor, is of the same genetic constitution as the New Zealand Red, a brown-eyed breed with a reddish yellow coat. The white doe was a daughter of pure-bred registered stock, which had been winning prizes consistently in rabbit shows and manifested all the characteristics of the New Zealand White breed. Therefore it seemed logical to assume that this doe was genetically brown-eyed,

although production of brown pigment was inhibited by the epistatic relationship of albinism. All of her gametes might be expected to transmit brown eye, which, as we have seen, is dominant over blue eye, and so only brown-eyed young might be expected from a mating with a blue-eyed chinchilla animal. But if the albino parent did not exert any influence on the eye color of her offspring, then these might be expected to be of two classes, (1) blue-eyed and dark-coated and (2) brown-eyed and pale-coated. These two classes (and no others) actually resulted. They are included in the progeny of mating 1, table 3. The brown-eyed young in this case were

TABLE 3—Tests for linkage between dark coat and blue eye in matings of animals heterozygous for both characters with animals possessing neither. Expected numbers in parentheses.

Mating No.	Heterozygous parent	Homozygous parent	Blue-eyed dark-coated chin. young	Brown-eyed young	Totals
1	♂ $ch^3 ch^1$	♀ albino	28	27 pale chin.	55
2	♀ $ch^3 ch^1$	♂ albino	23	20 pale chin.	43
3	♂ $ch^3 ch^2$	♀ albino	17	18 light chin.	35
4	♀ $ch^3 ch^2$	♂ albino	30	40 light chin.	70
5	♂ $ch^3 C$	♀ albino or $ch^3 c$	47	35 full color	82
	Totals		145 (142.5)	140 (142.5)	285
	Dev. and P.E.		2.5 ± 5.7	2.5 ± 5.7	

notably paler in coat color than the pale chinchillas previously obtained. This is characteristic of pale chinchillas heterozygous for albinism, as shown by Nachtsheim. The albino gametes of brown-eyed rabbits of full color but heterozygous for albinism were shown to be incapable of transmitting brown eye in matings with dark chinchillas. Eleven offspring have been so produced, of which six were blue-eyed dark chinchillas and five were brown-eyed full colored.

Albino gametes from two other sources were likewise found to be incapable of influencing eye-color. Male No. 116 belonging to a pure bred, albino angora race has been used extensively in these experiments. Little is known of his pedigree. All 18 of his offspring by blue-eyed chinchilla does were blue-eyed.

A male Himalayan albino was mated to a blue-eyed chinchilla doe, and the offspring resulting were all blue-eyed dark chinchillas not different in appearance from heterozygotes with true albinism.

It was now evident that none of the available sources of albino gametes were able to transmit brown eye. However, blue eye being a recessive, even a moderate degree of linkage between blue eye and albinism would make possible the carrying along of such a gene for generations without its manifestation in full-color combinations.

TEST FOR LINKAGE BETWEEN BLUE EYE AND DARK CHINCHILLA

The best test for linkage is made by mating the doubly heterozygous combination with the double recessive. In this case albinos (male 116 and his offspring by albino female 119) were used as the double recessive class, since they carry neither dominant (brown eye or colored coat), it

being assumed for the purpose of the experiment that these characters are possibly due to different genes.

There were available as double heterozygotes three classes of brown-eyed rabbits. The first class consisted of brown-eyed chinchilla animals (ch^3ch^1) produced in mating 6 (table 2) in crosses of blue-eyed dark chinchillas (ch^3) with brown-eyed pale chinchillas (ch^1).

A second class of double heterozygotes resulted from the mating of blue-eyed dark chinchillas (ch^3) with brown-eyed light chinchillas (ch^2). The 31 animals which composed this group (ch^3ch^2) were indistinguishable in appearance from the class just previously mentioned (ch^3ch^1).

A third class of double heterozygotes resulted from matings of blue-eyed chinchillas (ch^3) with brown-eyed animals of full color (C). In a few cases, blue-eyed chinchilla does heterozygous for albinism (ch^3c) were used as the double recessive parent in matings in which the doubly heterozygous parent was full colored. Such matings would allow crossovers as regards eye color to be detected equally well. See mating No. 5, table 3.

Most careful attention has been given to the (ch^3ch^2) and the (C ch^3) matings because in these, two other variations were involved, namely yellow or rusty back and minor variations in eye pigmentation to be discussed later in this paper. In conducting these experiments all precautions were made to insure the raising of entire litters. In so far as possible only three or four young were left with each mother. All in excess of this number were transferred to a foster mother at birth. In spite of these precautions there has been some loss, which makes the record incomplete.

Table 3 shows that in a total of 285 backcross young no crossovers have been observed between dark coat and blue eye, which were originally associated in the blue-eyed dark chinchilla ancestor of the heterozygous parent. All of the 145 dark chinchillas are blue-eyed, and all of their 140 sibs are brown-eyed, whether pale coated, light coated, or of full color, according to the nature of the cross. The segregation of blue eye from brown eye is clearly on a 1:1 basis.

In the early classification of the young produced by mating 1 (table 3), 12 pale-coated individuals were recorded as blue-eyed and accordingly as crossovers. But subsequently there developed in their eyes enough pigment to give them a very light grayish, rather translucent brown color. They have accordingly been classified as brown-eyed. An additional reason for so regarding them is the fact that no complementary crossovers have been observed, that is dark coated brown-eyed individuals. A final test of the correctness of this interpretation is afforded by a mating with a blue-eyed, dark chinchilla sib. If the suspected individual is genetically blue-eyed, only blue-eyed offspring would result. Actually, however, among ten offspring, two very light brown-eyed chinchillas resulted which could only have come from a brown-eyed gamete contributed by its pale chinchilla mother.

In support of the view that blue eye is completely linked with dark chinchilla (or is a characteristic feature of the allelomorph ch^3) may be recalled

the fact that two different strains of albinos derived from brown-eyed families possessing full color have failed to transmit brown eye when mated with blue-eyed chinchillas. Two other albino strains of different origin have likewise failed to transmit brown eye. The question now arises as to the probable relationship of blue-eyed chinchilla to gray-eyed chinchilla described by Castle. Is it another allelomorph of the albino series? Unfortunately the race previously studied by Castle is now extinct and it is not possible by breeding tests to answer this question. But evidence is available from other sources which suggests that blue-eyed chinchilla is not a new allelomorph.

VARIATION IN EYE COLOR

Thus far in this paper, the term blue eye has been used in a rather general sense to include all eyes, characterized by absence of pigment from the anterior surface of the iris. Such appears to be the eye character of the wall-eyed White Dutch and Vienna White races described by Castle (1925) and Punnett and Pease (1925) in which the absence of pigment from the front of the iris is complete. In the blue eye of chinchilla rabbits the general appearance is similar but the absence of pigment is only partial, there being always a small amount of anterior pigment present even in the clearest blue eye of this race (see figs. 10, 11 and 12).

Variation in the amount of such pigment had been recognized from the beginning of this study, but its theoretical importance was not appreciated until the appearance of certain individuals, which were first recorded as blue-eyed dark chinchillas, but which at maturity resembled brown-eyed heterozygotes ($ch^3 ch^1$). At first this was considered a possible result of an error in recording but the breeding behavior of two of these animals has shown the record to be correct. The eye color of such individuals may be designated pseudo-brown. When they are mated with albinos or with blue-eyed chinchillas they produce only blue-eyed young, some of which, however, usually become pseudo-brown with maturity, while others remain obviously blue.

For the purpose of more intensive study, this brown contamination of the blue eye, which seems to vary continuously, may be classified into five grades, varying from a nearly clear blue to the darker pseudo-brown types (see fig. 26). In the latter the spots of pigment become so numerous as to appear practically continuous. In grade 5 the entire surface is covered, except that here and there small pinpoint dots of blue are usually visible, which pigment has failed to cover and these occur especially in the dorsal sector of the iris. In figures 12, 13, 14 and 15 are shown photographs of some of these types.

It is impossible to classify doubtful cases with certainty until the animals have attained an age of at least eight weeks. Many have been raised to maturity and among them there has been found no change after twelve

TABLE 4—*Variation in eye color among offspring of matings involving various grades of iris pigmentation as indicated.*

Mating No.	Parents	Grade of blue-eyed offspring					Grade of brown-eyed offspring			
		1	2	3	4	5	1 Very Lt. Brown	2 Lt. Brown	3 Md. Brown	4 Dark Brown
1	Grade 1 modified blue-eyed females (ch ³ c) x albino ♂ 116.....	5	7	..	..	..	..	..	..	..
2	Grade 3 modified blue-eyed females (ch ³ c) x albino ♂ 116.....	3	9	14	5	..	..	..	..	..
3	Grade 4 modified blue-eyed females (ch ³ c) x albino ♂ 116.....	..	6	16	6	2	..	..	..	..
4	Dark brown-eyed chinchilla females (ch ³ ch ²) x albino male 116.....	..	4	17	1	1	..	14	10	12
5	Dark brown-eyed chinchilla ♂ 4713 (ch ³ ch ²) x albino females.....	..	8	10	..	..	..	6	6	1
6	Light brown-eyed dark chinchilla males (ch ³ ch ¹) x albino females.....	10	12	3	..	..	11	10	..	..
7	♂ 34M1 (C ch ³) x (ch ³ ch ³) or (ch ³ c) females of grade 1 blue-eye.....	2	5	..	..	..	..	3	2	..
8	♂ 34M1 (C ch ³) x (ch ³ ch ³) or (ch ³ c) females of grade 2 blue-eye.....	1	4	4	..	..	..	1	3	..
9	♂ 34M1 (C ch ³) x (ch ³ ch ³) or (ch ³ c) females of grade 3 blue-eye.....	2	7	5	1	..	..	3	3	..
10	Grade 3 blue-eyed dark chinchilla fe- males x grade 1 blue-eyed dark chinchilla ♂ 71N2.....	4	10	3	..	..	..	..	..	..
11	Grade 4 blue-eyed dark chinchilla fe- males x grade 1 blue-eyed dark chin- chilla ♂ 71N2.....	4	14	11	3	..	..	..	..	..

weeks. Two families which have manifested a tendency for pigment production throughout the life of the individual have been excluded. These may be genetically the same but influenced by other modifying genes. All individuals have been kept until there was no indication of further change. This study is not adequate to demonstrate completely the manner of inheritance of this variation. However, from the standpoint of the nature and effect of each of the albino allelomorphs it is important to know whether such variation is inherited and, if so, whether it is associated exclusively with the dark chinchilla allelomorph (ch^3).

Considerable data upon this variation have been obtained from the crosses already described. Among the dark chinchilla does mated to albino male 116 were several which were classified as grade 1. Of the twelve offspring so obtained (mating 1, table 4) seven were of grade 2 and five were of grade 1 which is a fair approximation to a 1:1 ratio of grades 2 and 1. This indicated not only that the albino male was heterozygous for some gene inducing grade 2 pigmentation, but most important that these modifying genes unlike true brown eye may be carried by the gametes of an albino in which sort of individual they produce no visible effect.

Some indication of the genetical difference between grades 3 and 4 was first found in the distribution of the offspring derived from females of these grades when mated with male 116. In mating 2, table 4, is shown the distribution of the offspring of grade 3 blue-eyed dark chinchilla does with respect to grade of eye color, and in mating 3 is similarly shown the distribution derived from grade 4 females. The mode is found to be class three in both cases, but the range starts and ends one grade higher in the case of the offspring of grade 4 does.

A similar distribution was found in the offspring of the heterozygous ch^3ch^2 females mated to male 116. The parents of these females were two dark chinchilla does of grade 4 modified blue eye and a light chinchilla male of medium brown eye, the angora rex previously described. The F_1 brown-eyed offspring were dark and medium in modification, but it happened that only the former produced offspring.

The progeny of these have been classified in mating 4, table 4, according to the grade of eye color. A similar degree of variation was found in both blue-eyed dark chinchillas and in brown-eyed light chinchillas (see figs. 16 and 17) although the gradations are not as clear in the case of the brown eyes of the light chinchillas. The variation here is due merely to the degree of intensity of the brown, making the certainty as to whether an individual belonged in one class or in the next above sometimes questionable. The extreme classes, however, were never in doubt nor was there ever a question involving more than two adjacent classes. In the reciprocal cross, that of a ch^3ch^2 male, comparable differences were also found in eye color of the ch^3c and ch^2c young as shown in mating 5 of table 4. The range includes for the most part only grades 2 and 3. The smaller range may be due to chance but more likely is to be explained on the basis of the genetically lower grade of iris pigmentation of the albino females.

The genes responsible for this variation also seem to influence the intensity of brown in the eye of pale chinchillas, as shown by the matings of ch^3ch^1 males with albino females in table 3, mating 1. The light brown eye and the pedigree of the males indicated them to be of a very low grade of iris pigmentation. The albino females used were of the same stock previously described and therefore probably of a low grade. The offspring have been classified according to grade of eye color in mating 6, table 4. The distribution here includes grade 1 blue eye not found in the other matings and also a group not previously described, a very light brown. This type is of special interest because of its blue appearance at 4 to 6 weeks of age. This, together with the fact that at the time of first appearance crossover classes were being sought between blue eye and the albino series, suggested to me that it might be a blue-eyed pale chinchilla (ch^1). Subsequently, however, the development of additional pigment showed it to be a very light brown eye, not a blue as found in dark chinchilla.

A similar and even lighter modification of brown eye was observed among the F_2 segregates from a medium brown-eyed light chinchilla crossed with albino female 119. Two of these individuals were first considered to be the result of the union of crossover gametes with albino non-crossover gametes. The crossover of blue eye in this case would have been from albinism to light chinchilla (ch^2). The subsequent development of further pigment, however, resulted in a very light brown eye at the age of three months. Later four other individuals of this type appeared out of fourteen sibs, making it improbable that these arose as crossovers, especially since each individual must have arisen as the union of two crossover gametes, making a total of twelve crossover gametes from this one cross involving an F_2 population of only twenty-three individuals in all. The most probable explanation of these cases seems to be a lack of modifying genes, which lack makes a genetically brown eye look blue at an early age. Such an interpretation is not illogical if we remember that albino female 119 carries a low-grade iris pigmentation and that the light chinchilla parent was classified as having a medium brown eye.

The brown eye of full-colored rabbits is also influenced in the same manner as demonstrated by the matings of full-colored male 34M1 (Cch^3) previously described (see figs. 18 and 19). He was light brown-eyed, the son of female 118 a grade 2 blue-eyed dark chinchilla and was sired by a dilute sooty yellow ($Cdea$) male. The latter male was apparently of a low-grade iris pigmentation, since all of the F_1 s were of a low grade. The results of mating 34M1 with dark chinchilla females of each of the grades 1, 2 and 3 eye color are shown in matings 7, 8 and 9 of table 4. The numbers are small but the same tendency to segregate several shades of brown is apparent.

That light brown eyes of full-colored rabbits are genetically the equivalent in grade of iris pigmentation to low grade blue eyes of dark chinchilla is further indicated by matings of two light brown-eyed full-colored daughters of 34M1 with grade 1 blue-eyed dark chinchilla male 71N2. Eight off-

spring have been raised to twelve weeks of age, five of which are full colored with a light or very light brown-eye and three are dark chinchillas, two of grade 1 blue-eye and one of grade 2 blue-eye.

Similarly very light brown-eyed pale chinchillas heterozygous for albinism have been mated to grade 1 blue-eyed dark chinchilla male 71N2 (also heterozygous for albinism). There have been produced seven blue-eyed dark chinchillas of grades 1 and 2, eight very light brown-eyed pale chinchillas and three very light brown-eyed dark chinchillas, undoubtedly heterozygous for dark and pale chinchilla (ch^3ch^4).

Corresponding matings of very light brown-eyed light chinchillas can not be shown at this time. Evidence that very light brown eye in this type of chinchilla is also genetically similar to low grade blue eye of dark chinchilla has already been presented, since neither a grade 1 blue eye nor a very light brown eye is found in matings 4 and 5 of table 4. They are found, however, as described above, in the F_2 from the cross of a medium brown-eyed light chinchilla male with albino female 119 known by previous breeding test to be genetically of low grade iris pigmentation.

An early attempt to formulate a working hypothesis led to the assumption of two pairs of genes having a cumulative effect upon the pigmentation of the front surface of the iris as the simplest possible explanation of the then known facts. As a test of this hypothesis, matings were made between grade 3 and grade 4 blue-eyed dark chinchilla does and a grade 1 blue-eyed dark chinchilla male, 71N2. Of the six grade 3 mothers used there is good reason from their pedigrees to think that five were heterozygous for both pairs of iris modifiers. The sixth doe could have been either homozygous for one pair of genes or heterozygous for both pairs. But as she produced only three young, her contribution affects the general result very little. The combined result (mating 10, table 4) approaches the 1:2:1 ratio to be expected from such matings, based on this hypothesis.

The progeny of the grade 4 does are classified in mating No. 11, table 4. The genetic composition of the mothers may be assumed to have been either $MMM'm'$ or $MmM'M'$, on the basis of two pairs of genes having a cumulative effect, but in that case only two classes of young are to be expected, *viz*, grade 2 and grade 3. However, there have appeared in addition four grade 1 individuals and three grade 4 individuals which in no way could be classified with the other two groups. In order to derive four classes from such a mating it would be necessary for the grade 4 parent to be heterozygous for each of three separate genes and these matings may be considered as evidence that a Mendelian interpretation of this continuous variation must be based upon at least three pairs of genes.

It is significant that none of these types, grades 2, 3 or 4 appear to be completely dominant to grade one, nor do any of the matings made up to this time manifest a strictly "blending" type of inheritance. This is evidence of genetic heterozygosity of the animals used and that the genetic factors involved are somewhat numerous. It may account, together with possible

heterozygosity of dark chinchilla with light or pale chinchilla, for the difficulty which fanciers report in obtaining true-breeding brown-eyed chinchillas.

If the factors involved are numerous, it is quite probable that the grade 3 and grade 4 individuals used in these experiments are more or less heterogeneous as to their genetic constitution and therefore the ratios obtained in table 4 are not valid in any precise determination of the genetic factors involved. I can not therefore attach great importance to any formulations of factorial composition at this time. Matings which will give a more precise basis for such a formulation are now in progress and may be reported in the future.

It was shown above that albino male 116 was genetically of a higher grade eye color than grade 1. It is interesting to note that this fact is substantiated also by the distribution of his offspring from grade 3 and grade 4 does as compared with those obtained from male 71N2, a grade 1 blue-eyed dark chinchilla who was also mated with grade 3 and grade 4 females. (Compare matings 2, 3 with matings 10 and 11 of table 4.)

While blue-eye appears to be essentially an effect of the dark chinchilla allelomorph, the data presented above constitute evidence that it is secondarily influenced by modifying genes responsible for the presence of more or less pigment in the front wall of the iris. These genes appear to be transmitted independently of the dark chinchilla gene, since corresponding reductions of pigment intensity are found in light and pale chinchilla segregates and in full-color segregates produced in the same litters as the dark chinchilla young.

HETEROCHROMIA IRIDIS IN DARK CHINCHILLAS

A number of cases in both man and animals are on record in which the iris of one eye (or parts of it) differ in color from the iris of the other eye. In man two types have been recognized, one which is pathological and the other hereditary. Bond (1912) has discussed the latter type in some detail. Perhaps there is no better known example than that which occurs in the White Dutch and Vienna White races described by Castle and by Punnett and Pease. The heterochromic condition in these races appears more or less characteristic of certain degrees of white spotting or marking.

During the course of this study upon eye color, several cases of mosaic iris have come to my attention which are of interest both from their relation to this particular problem and also from the point of view of mosaics in general.

Among certain matings of blue-eyed dark chinchillas with pale chinchillas heterozygous for albinism have appeared two heterochromic individuals. One of these, dark chinchilla doe 86I3, was first recorded at one month of age as having a gray-blue eye with a dark brown sector in the upper posterior portion of the iris. The bluish gray sector of this eye gradually became brownish until at maturity it was classified as a very light brown. When this doe was mated with albino male 116, four dark chin-

chillas and three pale chinchillas were produced, showing the heterozygous nature of the mother. None of these young showed any indication of heterochromia.

The second heterochromic individual, dark chinchilla (ch^3c) doe 27R1 (see fig. 28) arose from the same father mated with an unrelated pale chinchilla doe (ch^1cA). Her left eye was first recorded as a grade 2 blue eye but later attained to grade 3. The right eye was like the left in the dorsal half of the iris, but the remainder was unmistakably a grade 4 when first classified and at maturity was an intense and uniform brown, almost black. By pedigree this animal was known to be heterozygous for albinism, which fact was confirmed by a mating with male 71N2, a blue-eyed dark chinchilla of grade 1 eye color. This mating resulted in three out of nine offspring which were albinos. None of these were heterochromic.

The case of 86I3 might be regarded as a mosaic of the type described by Castle (1929) of the blue and black mosaic in the rabbit, or the case described by Pincus (1929) of a chocolate and black mosaic in the mouse. In both of these cases the mosaic individuals were found to be heterozygous, the one for black and blue, the other for brown and black. My animal 86I3 was found to be heterozygous for dark and pale chinchilla and the mosaic eye might be explained as a mosaic condition of the brown and blue eyes of these respective types.

The second case, however, that of 27R1 can not be explained in this manner. She is indeed heterozygous for albinism, but albinism, as we have seen, does not transmit the allelomorph eye colors, but only their modifiers. While a portion of this eye at maturity is to be described as very dark brown or black, previous records show that it has unmistakably behaved throughout its development as a modified blue eye, one portion of which is a grade 5 or pseudo-brown, while the other portion and the left eye are of grade 3. Hence the mosaic in this case is not of albino allelomorphs, but of the modifiers, which we have seen are transmitted by albino gametes as well as by chinchilla and full-color gametes.

Referring again to the mosaic eye of female 86I3 it will be noted that the self-colored eye was first recorded as a gray-blue and was later classified as a very light brown. This slow transition from gray-blue to very light brown is characteristic of those eyes of light (ch^2) and pale (ch^1) chinchillas known to bear the minimum of iris modifiers. The same situation is also found among ch^3ch^1 heterozygotes which by pedigree could only bear the minimum of iris modifiers. The pedigree of 86I3 was not such as to definitely classify her as of this type, but her father was a grade 3 male which has been the sire of several grade 1 blue-eyed dark chinchillas and the mother was recorded as having a medium to light brown eye. A litter mate of 86I3 is also recorded as being a grade 1 blue-eyed dark chinchilla. It seems probable therefore that the heterochromic eye of 86I3 is also to be explained like that of 27R1 as a mosaic distribution (somatic segregation) of the genetic factors which modify the intensity of pigmentation of the front surface of the iris.

A third heterochromic iris is pictured in figure 27. The two eyes of this animal, dark chinchilla female 14A1, were similar to each other. Each shows a sector of the blue eye of White Dutch at the bottom, above which on each side is a sector of pseudo-brown iris of dark chinchilla which gradually gives way dorsally to a sector of grade 3 pigmentation. The parents of this animal, which is a dutch marked rabbit of grade 9 (see plate 1, Part 1, this publication), were both nearly self-colored, showing only white toenails on the front feet as evidence of the White Dutch which they seem to have carried. The mother in this case was a half sister of 27R1 by the same sire and both parents were of a grade 3 eye color.

At the present time adequate test has not been made to determine whether or not this mosaicism extends to the germ cells. However, it seems hardly possible that three individuals as closely related as these three could bear such a striking irregularity by chance alone. The presence of the White Dutch sector of the iris in 14A1 is explicable from the homozygosity of White Dutch in this animal (Castle 1926). From this animal alone we might attribute the heterochromic condition of the dorsal part of the iris to the influence of the White Dutch gene. However, 27R1 and 86I3 are not known to carry this gene as is also the case of 81B1, their sire, so that the mosaic condition of their eyes can hardly be due to its influence. Somatic or germinal, an adequate explanation of this irregularity seems to be directly dependent upon an understanding of the inheritance of the normal variation in pigmentation of the front surface of the iris. Experiments are now in progress which it is hoped may throw additional light upon both of these problems.

HISTOLOGICAL STUDY OF GRADES OF EYE COLOR

It has been shown that the blue eye of dark chinchillas may be masked in varying degrees by the development of brown pigment in the anterior wall of the iris. A corresponding development of pigment in the other types of chinchillas or in animals of full color causes similar variation in the depth of eye color, though it does not change its quality, since all such types are brown-eyed. It may be assumed that the same genetic agencies are operative in both cases since segregation occurs in like fashion of different intensities of brown eye and of different degrees of modification of blue eye.

A comparative histological study has been made of the pigmentation of the iris in eyes of various types including full color in both dark and light shades, pale chinchilla (ch^1) in both dark and light shades and dark chinchilla (ch^3) in grades 2 and 4 of the modified blue eyes. See figures 20 to 25. No study has yet been made of the eyes of light chinchillas (ch^2).

In preparing the eye for histological examination, the procedure has been to remove and fix it entire. A slight incision was made with a pair of fine scissors through the sclera and choroid into the posterior chamber so that the fixative may readily penetrate to the interior of the eye. It was not found practicable to imbed the entire eye because of its large size. There-

fore an incision was made in the region of the ciliary body, opposite the third eyelid, for the purpose of later orientation at dissection. Several fixatives have been tried, including Zenker's and Bouin's fluids and formol-acetic. The last named, suggested to me by Dr. A. B. Dawson, consists of 95 parts of 10 per cent formalin and 5 parts glacial acetic acid. This appears to cause a slight degree of swelling, enabling better perception of the processes of the fibroblasts.

When the eye was removed from the fixative, it was run up to 70 per cent alcohol. Before going farther the eye was divided into two parts, an anterior portion including the cornea, iris, ciliary body and a small portion of the retina, choroid and sclera; and a posterior part including the most of the retina, choroid and sclera and the optic nerve. The anterior portion was then divided into four sectors, which were imbedded separately in paraffin by the usual histological methods and sectioned in such a way as to give radial sections of the iris. The dorso-ventral radii do not make possible an accurate estimate of the pigmentation of the iris because in both light chinchillas and pale chinchillas there is a distinct difference in the pigmentation of the dorsal and ventral sectors of the iris. Accordingly the lateral segments were the ones studied. Sections were cut 10μ thick and were lightly stained in Delafield's Hæmatoxylin and counterstained with eosin.

FULL COLOR

In the eyes of full-colored animals of both dark-brown and light-brown shades, the two layers of retinal epithelium are so filled with pigment that all details of cell structure are obscured (see figs. 20 and 21).

In the iris of the dark-brown eye the stroma, which consists of a vascular loose connective tissue, has many branched chromatophores scattered about irregularly but especially concentrated at the front surface of the stroma in such a way as to form a thick wall of pigment. Most of the branched chromatophores of the stroma mass are less intensely pigmented than those of the anterior margin, which makes them appear brownish instead of intense black as are those at the margin.

In full-colored animals having a light-brown eye (see fig. 21) there is a smaller number of pigmented chromatophores at the surface of the iris and a less intense pigmentation in agreement with the macroscopic observations on living animals. Branched chromatophores in the stroma mass are comparatively rare. The retinal epithelium and choroid are about the same as in animals with a dark-brown eye.

BLUE-EYED DARK CHINCHILLA

In blue-eyed dark chinchillas of both grade 2 and grade 4 (see figs. 22 and 23) the retinal pigmentation is slightly less than in animals of full color. Here and there can be distinguished nuclei and other cell structures which in the eyes of full-colored animals are entirely obliterated. This is represented in the photomicrographs of Plate 5 by the slightly broken appearance

of the retinal epithelium, whereas in a full-colored iris it is a solid black. The stroma contains only occasional branched chromatophores within which the pigment appears less dense and less closely packed than in the eyes of full-colored animals. Both grades 2 and 4 have branched chromatophores at the surface in which the pigment is intense and closely packed. These chromatophores tend to clump and grade 4 differs from grade 2 only in the tendency for the clumps to be larger, more numerous and to run together. The smaller and less numerous clumps of grade 2 leave large unpigmented areas which appear blue in the living animal.

PALE CHINCHILLA

This type (see figs. 24 and 25) differs from full color and dark chinchilla in having a marked reduction of the pigmentation in the retinal epithelium and choroid. Nuclei and other details of cell structure may readily be seen. The light-brown eye of pale chinchilla differs from very light-brown eyes of the same type chiefly in the greater number of branched chromatophores on the front wall of the iris and in the greater intensity of their pigmentation. In figure 24 the intensity of pigment at the front surface of the iris appears nearly as great as in the blue eyes of figures 22 and 23. In no case, however, is the pigmentation of the pale chinchilla as intense or as compact as in dark chinchilla, but it is more diffuse and brownish, and as shown in figure 24 the chromatophores appear to stand on end rather than flat against the surface. In the light-brown eye of pale chinchillas the intensity of the pigmentation is about the same at the front surface of the iris as in the retinal epithelium.

On the whole the microscopic examination substantiates the view that the variation found in the intensity of pigmentation at the front surface of the iris in full-colored and in pale chinchilla animals is comparable to the variation found in the front surface of the iris in dark chinchilla animals. This anterior pigmentation in blue-eyed animals alters blue to gray or pseudo-brown, but in animals of full color and in pale chinchillas it merely intensifies light brown to dark brown. The influence of the albino allelomorphs upon the pigmentation of the choroid and retinal epithelium is a graded one decreasing regularly from full color to pale chinchilla. But the variation in iris color found in the series is discontinuous in the case of the dark chinchilla allelomorph which is practically without anterior iris pigment in individuals with clear blue eyes. Yet in the pseudo-brown modifications of blue eye (grades 2 to 5) a certain amount of anterior iris pigment is found. Variation in this is independent genetically of the albino series.

RUSTY BACK

Yellow appears to be consistently absent from the coat in the lower members of the albino series (Himalayan and complete albino). Wright (1925) found the same to be the case in the guinea-pig and it suggested to him that yellow was due to a physiological process which requires for its realization

a higher threshold of the fundamental process necessary to all pigment formation. Castle (1926) in his study of the chinchilla rabbit noted the fact that "in the chinchilla the color factor scarcely reaches the threshold for the production of yellow pigment." In pale chinchillas, yellow is entirely absent and so far as I have observed the same is true of light chinchilla.

In dark chinchillas, yellow is commonly limited to the first pelage in which it develops in a well-defined dorsal area. It is this fact together with the rusty appearance of the individuals showing most yellow which has suggested for it the term "rusty back." This rusty or yellow area extends from nose to tail, and downward on the sides to a line passing from the eye through the hip joint, viewed as the animal sits at rest. Below this line on each thigh is found a small spot of yellow, but in the remainder of the coat yellow is lacking. In full-colored agouti rabbits two inguinal spots of yellow are found, but these do not occur in chinchilla rabbits. Coats, subsequent to the first, develop less yellow pigment, and at maturity the agouti banding of the less intense rusty young becomes a clear white. Pelts which are intense rusty when young may often be distinguished in the adult coat by an ashy-gray agouti band in place of the pure white.

Variations in the intensity of yellow in rusty back were early recognized and it was found convenient to classify them into four more or less arbitrary types—intense, medium, trace and non-rusty. No special matings have been made in the analysis of rusty, but careful records have been kept of the grade of rusty involved in all chinchilla matings and their progeny, and on these records this discussion is based. While they are not as conclusive as could be desired, they show the more salient hereditary features of this character and especially its relation to the albino series.

Among the early matings of the heterozygous dark chinchillas ($ch^3 ch^1$), were eight in which both parents showed a trace of rusty in the baby pelage. The results of these matings are summarized in table 5, mating 1. Approximately one-fourth of the offspring were homozygous pale chinchillas which showed no rustiness. About a third of the rusties were *medium* and two-thirds showed a *trace* or were *non-rusty*. The entire series might be interpreted as a 1:2:1 distribution of *medium*, *trace* and no *rustiness*, respectively. The absence of rustiness from the pale chinchillas is very significant, indicating that it can not develop in that genotype, and its distribution in the dark chinchillas suggests that two doses of rustiness produce a stronger effect than one.

Additional evidence of this is found in matings 2 and 3, table 5, in which several matings are combined of dark chinchillas showing a trace of rusty with albino ♂116. A similar distribution is here found, indicating that the albino male is probably a carrier of rustiness in the heterozygous condition, since some of the young show more rustiness than their mothers. The data have been divided to show separately the progeny from does heterozygous and homozygous for dark chinchilla. If homozygosity or heterozygosity of dark chinchilla is accountable for a part of the variation in rusty

TABLE 5—*Variation of the intensity of yellow among offspring of rusty back dark chinchillas.*

Mating No.	Parents	Rusty-backed offspring				Light or pale chin. offspring
		Intense	Medium	Trace	Non-rusty	
1	Dark chinchillas (ch ³ ch ¹) showing trace of rusty back	..	10	15	4	8 (ch ¹ e)
2	Dark chinchilla females (ch ³ ch ³) showing trace of rusty back x albino ♂ 116	..	..	7	6	..
3	Dark chinchilla females (ch ² e) showing trace of rusty back x albino ♂ 116	..	6	11	5	..
4	Dark chinchillas (ch ³ ch ³ or ch ³ e) showing trace of rusty	..	2	7	3	..
5	Total progeny in matings involving trace of rusty back. Expected numbers and P. E. in parentheses	..	18 (19 ± 2.5)	40 (38 ± 2.9)	18 (19 ± 2.5)	..
6	Medium rusty dark chinchilla ♂ 4713 (ch ³ ch ²) x albino females	..	4	9	1	17 (ch ² e)
7	Medium rusty dark chinchilla does (ch ³ ch ²) x albino ♂ 116	..	2	9	3	14 (ch ² e)
8	Trace of rusty dark chinchilla ♂ 52A1 (ch ³ ch ¹) x albino females	..	1(?)	7	8	17 (ch ² e)
9	Medium rusty dark chinchillas, mated inter se. . . .	..	..	4	3	..
10	Medium rusty dark chinchillas x trace of rusty dark chinchillas	6	24	25	8	..

back it might be expected that the homozygous females would produce the higher grades of rustiness. But deviation, small though it is, is in an opposite direction.

In mating 4 of table 5 are shown the progeny of several matings inter se of dark chinchillas which do not carry pale chinchilla (ch^1) and are of the grade trace of rusty back. Though the numbers are small, the resulting dark chinchilla offspring also appear to conform to a 1:2:1 distribution of medium rusty, trace of rusty and non-rusty.

When we combine the first four matings of table 5, which are considered to be inter se matings of individuals genetically of grade trace of rusty, the distribution fits very closely to the expectation of a 1:2:1 ratio, expected numbers and probable errors being shown in parenthesis.

Matings 5 and 6 of table 5 show again the offspring produced by mating the F_1 heterozygous dark chinchillas ($ch^3 ch^2$) of table 3 with albinos, the young being this time classified according to presence or absence of rusty back and its grade when present. Again we observe an approximation to a 1:2:1 distribution, suggesting a cumulative effect of a gene or genes for rustiness.

As pointed out above, male 116 seems to be heterozygous for rustiness. Mated with non-rusty females a 1:1 ratio is to be expected of rusty to non-rusty. Mating 7 shows such a distribution with only one doubtful darker individual recorded several days after death.

Further evidence that genetic differences affecting the intensity of rusty back are transmitted through albinism is afforded by the data shown in mating 8, table 5. Three albino females have been mated with heterozygous ♂ 52A1 ($ch^3 ch^1$), also graded as having a trace of rusty. Especially interesting are the offspring of ♀ 83A6. Dr. Castle has pointed out to me a variation in the intensity of red in certain New Zealand Red rabbits and also in ordinary gray rabbits which corresponds with the distribution of yellow in rusty back. Fanciers consider the more intense red types of the New Zealand Red the most desirable. This doe 83A6 was descended from prize winning New Zealand White stock and the white, being an albino form of the red, might be expected to transmit some sort of red or yellow intensifiers. She did transmit a higher grade of rustiness to her offspring than did the other albino does.

If the albino is able to transmit rusty back it seems reasonable to expect that light and pale chinchilla would also be able to carry it, although unable to manifest it. Evidence of this is found in the fact that none of the pale chinchilla offspring of matings 1, 5, 6 and 7 are rusty, while the dark chinchilla offspring of the same parents fall into a regular Mendelian distribution of rusty to non-rusty. Proof that pale chinchilla may carry rusty has been obtained by mating one of these homozygous pale chinchillas ♂ 86F1 with a dark chinchilla doe showing a trace of rustiness when young. Eight offspring were obtained, all of which were medium rusty. By a previous mating of the same doe with albino ♂ 116, six offspring had been produced, four of which showed a trace of rustiness while two were non-rusty.

Three other types of mating are of interest in this study. In mating 9, table 5, is shown the distribution of offspring of medium rusty dark chinchillas mated inter se. It approximates a 3:1 ratio of rusty to non-rusty with a deviation possible in random sampling, and includes three individuals having a rusty back of higher grade than either parent.

Much larger numbers are available from matings of medium rusty by a trace of rusty (see mating 10, table 5). These also show a range including intense rusty young. The probable error is such that the deviation from a 3:1 ratio in this case can hardly be due to chance. This together with the fact that medium rusty does not breed true but throws both higher and lower grades of rustiness suggests that the medium rusty condition may be a heterozygous one or due to more than one pair of genes perhaps acting in a cumulative manner.

If we assume the medium rusty individuals used in mating 10 to be heterozygous for each of two such genes and the individual bearing a trace of rusty to be heterozygous for one such gene, the expected ratio from such a mating would be in the proportion of 1:3:3:1 of intense, medium, trace and non-rusty, respectively. The total population in mating 10 approximates very closely such a distribution. Too much weight should not be attached to this, however, since on the basis of such an hypothesis random selection of twelve such medium rusty females would be likely to include some individuals which would be homozygous for one of these dominant genes as well as for the recessive allelomorph of the other. Study of the offspring of each female separately reveals only one individual in these matings which failed to produce either a non-rusty or an intense rusty, indicating it to be of the homozygous type. Only four young belong to her, which is too few to place her definitely as of this type.

Two matings were made of intense rusty chinchillas with those showing a trace of rusty. From them two intense, five medium and two having a trace of rusty resulted. The numbers are small, but resemble the 1:2:1 ratio to be expected if the intense rusty parent were homozygous for one pair of the dominant genes responsible for rusty back and heterozygous for the other pair and the other parent bearing the trace of rusty were heterozygous for only one of these dominant genes.

It is also interesting to note that among some forty dark chinchilla offspring of male 34M1 ($C\ ch^3\ Ee$) which were raised, all showed some rustiness, whereas four young produced in the same matings, and which genetically must also have been dark chinchillas, though they were in fact white coated (ee) animals, showed no trace of yellow, indicating that the expression of rusty back is dependent upon the presence of E , that is upon some physiological process which is lacking in non-extension of black (e). Nine other white-coated or non-extended dark chinchillas ($ch^3\ ee$) have been produced from the wide-banded chinchilla race presently to be described. These young also show no rustiness, though a high degree of it was manifested by their parents.

Although it has not been possible up to this time to carry the experiment far enough to determine the exact mode of inheritance of rusty back, the facts enumerated indicate not only that rusty back in the rabbit is due to a physiological process which sets in subsequent to the production of a color base, but that this subsequent process is dependent upon at least two genes inherited independently of the albino series. It also appears to be a process interrelated with the production of black pigment, such as is induced by the extension gene. In view of the fact that intense yellow pigment distributed similarly is found in full-colored agouti rabbits and in red ones, rusty back may be regarded as due to an intensifying gene which is effective to produce yellow at a threshold otherwise not reached by the chinchilla color gene.

RELATION OF RUSTY BACK TO EYE COLOR

Rusty back appears to be similar in type of inheritance to the modifications of blue-eye color. Wright (1925) in his study of the albino series of the guinea-pig found a tendency for the production of yellow pigment to interfere with the production of black. Such an effect has not been observed in the rabbit. A slight reduction of the intensity of black has been observed in non-agouti dark chinchillas which by pedigree were heterozygous for the extension gene (Ee) and so were capable of producing yellow young. Such individuals may be genetically rusty, though unable to show it because of the epistatic relationships of non-agouti. Deficiency in black on the part of chinchillas can not be ascribed to the production of yellow as an alternative process since the color gene does not reach the threshold for the production of yellow, except in the production of rusty back. Up to this time it has not been possible to test such non-agouti individuals for transmission of rusty back but if this character were due to an alternative process having a tendency to reduce the intensity of black, it seems possible that rusty backed chinchillas might have less pigment in the eye than non-rusty individuals. A comparison of eye color and rustiness in 158 dark chinchillas produced in these experiments shows no correlation to exist between the variations in rusty back and the variations in anterior pigmentation of the iris.

CHINCHILLA COMBINATIONS

In the course of these experiments, most of the possible combinations of albino allelomorphs have been produced (see table 6). In completing the series, three crosses were made, in addition to those already described, from which all missing combinations were eventually derived except that of non-agouti pale chinchilla combined with Himalayan albinism ($aa\ ch^1\ c^H$). Schultz (1930) has described this as differing from the albino heterozygote only in the color of the eye, which is darker. In view of the study of eye color which I have made, it seems probable that Himalayan has no greater

TABLE 6—Combinations of albino series of allomorphs arranged in order of intensity of black pigment in coat, choroid and retinal epithelium. Presence of yellow in coat or its absence is indicated in column three. Color of iris at different ages and with or without modifiers is shown in columns 5 to 8.

Combinations of albino series	Black	Yellow	Pupil of eye by reflected light	Iris with maximum of modifiers	Iris without modifiers		
					Age 4 weeks	Age 12 weeks	Maturity
C — all combinations	Full intensity	On back and sides	Black	Very dark brown	Light brown	Light brown	Light brown
ch ³ ch ³	Full intensity	Rusty back	Black	Pseudo-brown	Clear blue	Slightly speckled blue	Slightly speckled blue
ch ³ ch ¹	Full intensity	Rusty back	Black	Brown	Bluish brown	Gray brown	Very light brown
ch ³ c	Full intensity	Rusty back	Black	Pseudo-brown	Clear blue	Slightly speckled blue	Slightly speckled blue
ch ² ch ²	Trace of sepia	None	Slightly black	Brown			
ch ² ch ¹	Trace of sepia	None	Slightly black	Brown			
ch ² ch ³	Medium sepia	None	Medium red	Brown			
ch ² c	Medium sepia	None	Medium red	Brown	Bluish brown	Gray brown	Very light brown
ch ¹ ch ¹	Medium sepia	None	Medium red	Brown			
ch ¹ c	Light sepia	None	Very red	Brown	Bluish brown	Gray brown	Very light brown
ch ¹ ch ³	Light sepia	None	Very red	Brown			
ch ³ ch ³	Extremities only	None	Pink	White	White	White	White
ch ³ c	Extremities only	None	Pink	White	White	White	White
cc	None	None	Pink	White	White	White	White

influence than complete albinism on pigment production when it enters as a recessive into combination with pale chinchilla. The difference noted by Schultz is more probably due to independent modifying genes. This conclusion is supported by a comparison of agouti combinations of both albino and Himalayan heterozygotes which have occurred among my crosses. Although the presence of agouti in the combination makes the detection of differences more difficult, none could be observed.

Combinations of light chinchilla with Himalayan albinism and with pale chinchilla were produced by two special matings. The first mating involved a Himalayan male which was known to be heterozygous for brown (b) and was also a possible carrier of a modifying gene inducing a grayish belly, somewhat similar to if not identical with that found in steel-gray rabbits. The offspring consisted of five young — three normal agouti and two non-agouti. The individuals of each of these types were respectively similar when young to agouti and non-agouti heterozygotes of true albinism. Two agouti and one non-agouti young were raised to four months of age and, except for a slight grayish belly on the non-agouti individual, appeared to be identical with albino heterozygotes of this allelomorph. Slight variations were observed from time to time in their coats, which were shown by later observations to be due to moult.

Combinations containing the allelomorph dark chinchilla (ch^3) are distinguishable from those which contain light chinchilla (ch^2) or pale chinchilla (ch^1) by the fact that they are darker and do not fade. The only known exception is found in animals heterozygous for yellow coat (Ee) which may resemble (ch^2) animals in depth of pigmentation. Chinchilla animals homozygous for (e) are white coated; apparently this same gene, which practically inhibits the production of black pigment when homozygous in chinchilla animals, also decreases the production of such pigment when heterozygous.

The heterozygous combination of light with pale chinchilla ($ch^2 ch^1$) was produced by mating a light chinchilla female ($ch^2 ch^2$) with a pale chinchilla male heterozygous for albinism ($ch^1 c$). The resulting offspring fell into two classes of three individuals each as regards the intensity of their pigmentation. The darker animals which show a tinge of sepia in their coats, distinguishing them from combinations containing dark chinchilla (ch^3), are undoubtedly ($ch^2 ch^1$) zygotes, whereas the lighter class consists of $ch^2 c$ combinations. A female of the darker class was mated with albino ♂116 and produced eleven young all pigmented and of two classes. Five are of the $ch^2 c$ heterozygous type, and six are of the $ch^1 c$ heterozygous type, this being the kind of segregation expected and in the expected 1:1 ratio.

The mother of these young, a non-agouti animal ($ch^2 ch^1 aa$), is very similar in appearance to non-agouti dark chinchillas which contain the allelomorph ch^3 , but with this difference, that her black coat shows a tendency to fade, becoming brownish, whereas that of a ch^3 rabbit does not.

The identity of dark chinchilla homozygotes ($ch^3 ch^3$) and heterozygotes ($ch^3 c$) has been established in test matings with albino male 116. The

two types are phenotypically indistinguishable. All non-agouti dark chinchillas heterozygous for albinism have been found to be apparently equal in intensity of black to full-colored non-agouti animals.

The non-agouti form of light chinchilla has been produced in the second generation from a cross of a light chinchilla male with albino ♀119 previously described. It is distinct from dark chinchilla, although the difference is slight, being most noticeable on belly and sides, especially at the base of the hairs or in the tips of the fur in the baby pelage, where the temperature influences are most effective.

Combinations $ch^2 ch^2$ and $ch^2 ch^1$ are very much alike in appearance, though the former is slightly darker, but it would be impossible to distinguish between them with certainty unless one had the aid either of individual pedigrees or of test matings with albinos.

Homozygous pale chinchillas ($ch^1 ch^1$) have been obtained in both agouti and non-agouti combinations from matings inter se of brown-eyed dark chinchillas ($ch^3 ch^1$). Heterozygotes for comparison have been obtained from matings with albinos producing ch^1c combinations. The homozygous type is much darker, as shown by Nachtsheim in the case of the "Marder" rabbit. It is scarcely distinguishable in appearance from the heterozygous light chinchilla combination (ch^2c), but the regional reduction of pigment, especially on the sides, characteristic of pale chinchilla is evident.

Whether Himalayan albinism is completely dominant over the unpigmented type has not been determined. In general, the relation of one albino allelomorph to another is one of incomplete dominance of the higher over the lower, which would lead us to expect Himalayan albinism to be more effective in a double than in a single genetic dose in causing pigment production.

GENERAL DISCUSSION OF EYE COLOR

The data presented in this paper constitute evidence of the existence in the rabbit of at least three chinchilla allelomorphs, with one of which (dark chinchilla) blue eye is associated. Whether blue-eyed dark chinchilla is to be interpreted as a seventh member of the albino series of allelomorphs in the rabbit, distinct from the gray-eyed dark chinchilla of Castle and the brown-eyed dark chinchilla of Nachtsheim, seems doubtful. The evidence presented above indicates as probable an interpretation of eye color in the chinchilla based upon the primary effect of albino allelomorphs plus the action of certain independent modifying genes.

In table 6 are arranged the combinations of the albino series of allelomorphs in order of intensity of black pigmentation of the coat as determined in this study. Presence or absence of yellow and eye color are also indicated. The order of intensity of pigmentation in the choroid and retina of the eye is the same as in the coat, and this is expressed in the color of the pupil, which ranges from black to pink, the last-named color being due to the blood alone, unassociated with pigment.

As regards iris color, the order of decreasing pigmentation is the same (as for the coat) when a maximum amount of anterior pigmentation is present. But in the absence of such pigmentation, as shown in table 6, there is a break in the series since the darkest coated chinchillas have the lightest blue eyes. This is due to a peculiarity of the chinchilla allelomorph, ch^3 .

A number of studies of the inheritance of eye color in man have been made, the results of which have been summarized by Davenport (1927) and Gates (1929). Several analogies may be noted between the inheritance of blue eye in man and in the rabbit.

The general conclusions reached by all investigations of eye color in man is that blue eye is recessive to brown. Blue-eyed parents have only blue-eyed children, but brown-eyed parents may have both blue-eyed and brown-eyed children. Exceptions are found, however, as in the studies of Boas (1919), Bryn (1920), Gross (1921), Winge (1921) and Dürken (1925) in which brown-eyed or duplex eyes have been observed among the children of blue-eyed or simplex parents. One explanation which has been offered is that parents classified as blue may actually be gray or bear some modifying genes which in a proper mating would result in eyes of a browner shade. Such a situation actually exists in the pseudo-brown eyes of the chinchilla rabbit already described.

Modifiers of iris color occur among human beings as well as in the rabbit. Bond (1912) has noted several types of iris pattern and the fact that their inheritance is complicated. Bryn (1920) found that no normal blue iris was devoid of brown pigment and Gates (1929) reports a similar variation among the people of Bergen, Norway, which ranges from very light blue to dark brown as do the eyes of chinchilla rabbits. In the Bergen population the situation was complicated by the presence of yellow iris pigment which is not found in the rabbit. Vogt (see Gates 1929) found that children of parents with intermediate eye colors "are often darker but may be lighter than either parent." Such darker-eyed children were explained by Winge as pathological, but they need not necessarily be assumed to be such if genes modifying iris pigmentation are assumed to be present, such as are found in the rabbit.

The relationship of hair color to iris color in man as compared with the chinchilla rabbit is of particular interest. Brownlee (1913) made a statistical analysis of their association, concluding that linkage does exist between hair color and eye color. Wright (1918) suggested that there are two kinds of dilution in eye color in man. One genetic factor reduces black both in hair and eye, allowing red hair to appear, while another factor reduces the pigmentation of the iris without affecting the hair. This is similar to the situation in the rabbit, except that red does not appear in the reduction of black pigment, so that the situation is not entirely analogous. The red and yellow haired albinos of Pearson are also variations not found in the albino series of rodents. They may, however, correspond to some genetic combination of a variation such as salmon eye of the guinea-pig (Gregory, 1929).

The light-haired brown-eyed or black-eyed combinations, several of which are reported by Holmes and Loomis (1909), are not explained by Wright, but may be explained on the basis of albino allelomorphs corresponding in nature to pale and light chinchilla.

In view of the known homologies of unit characters among the different rodents and the several similarities between the behavior of eye color in man and in the chinchilla rabbit, it may not be out of place to suggest the importance of more careful correlation of hair and eye color in future human studies.

WIDE TICKING

The coat of the rabbit as described by Salaman (1922) and Kleefeld (1929) consists of at least three types of hair: (1) long primary guard hairs; (2) shorter, finer and much more numerous secondary guard hairs; and (3) much shorter, very fine and numerous under-hairs or wool hairs. In the cotton-tail rabbit, in the jack-rabbit and in ordinary gray domesticated rabbits the primary guard hairs are entirely black except for the basal end which, like the fur of all dark-colored rabbits, becomes a blue next to the skin. The pattern of the secondary guard hairs consists of a black tip or apical band, a subterminal agouti band of red or yellow and a basal band of black which shades to blue at the base. The under hair in color pattern is a duplicate of the secondary guard hair but is shorter and very much finer in texture. If one examines a plucked sample of fur or blows into the fur of the living animal in which the fur has completed its seasonal growth, one finds that the length of the fine wool hairs is such that their tips attain only the basal margin of the agouti band of the secondary guard hairs. The tips of the fine wool hairs coincide with the black basal portion of the guard hairs before they shade off to blue, and thus form a line of demarcation between two agouti bands, one on the secondary guard hairs and the other on the wool hairs. Any change in the width of these bands affects the appearance of the coat.

In the European rabbit, several mutant types have appeared which affect the width of these bands. Non-agouti eliminates them entirely, while dark extension in the heterozygous condition narrows them, and when homozygous nearly or quite obliterates them. In wild rodents the agouti pattern is very common. Its variations from one species to another, or from one regional group to another, are considered adaptations of the animals for life in particular environments. For example, rabbits living in plains and deserts are light in color, blending well with the whites, yellows and grays of their surroundings, while rabbits of upland, wooded or humid regions are darker in harmony with their surroundings. Close examination shows that such differences are due either to a difference in the intensity of the yellow pigment, or to a difference in the proportion of yellow to black in the coat. Very often differences of both sorts are found.

Sumner (1930) in stating the results of his numerous studies of the inheritance of sub-specific characters in *Peromyscus*, extending over a period of fifteen years, points to the probable importance of natural selection in producing adaptive coat coloration. It is possible that gene variations, such as affect the width of the agouti banding in the domestic rabbit presently to be described, will account in part for geographic variation in the color of wild rabbits.

In 1929 among a lot of rabbits consigned for market purposes, the writer discovered a chinchilla doe which was markedly whiter in appearance than the normal chinchilla. Closer inspection showed that this animal was lighter because of an increase in the width of the agouti band of the secondary guard hairs and under hairs (see fig. 29). Unfortunately the ancestry of this animal was unknown, but she was saved and mated to a normal chinchilla. Eleven F_1 offspring from this mating were all normal with no discernible variation in width of banding. The peculiar female with two of her daughters and a son were brought by the writer to the Bussey Institution in 1929. An F_2 generation produced there consists of 74 normal individuals and 27 wide-banded ones, which is a close approximation to a 3:1 ratio. The wide-banded offspring, like their grandmother, have an agouti banding approximately twice as wide as that of the normal agouti coat, with a variation in width of band of only 1 or 2 mm. The narrow-banded individuals show little variation from the condition found in their narrow-banded ancestors. The backcross of the F_1 male with his mother has produced 16 narrow-banded or normal agoutis and 17 wide-banded. Wide-banded F_2 individuals mated inter se have produced 18 young, all wide-banded. Hence the character behaves as a typical Mendelian recessive. About 20 per cent of the data of the F_2 and backcross matings have been collected from animals at birth. It has been found that the wide-banded condition can be distinguished from the narrow at 1 to 3 days of age by a "bald" appearing spot upon the top of the head due, probably to the fact that the secondary guard hairs of this region are producing no pigment at the time and so the spot is devoid of *visible* hair. Later, as the under hairs begin to form their black tips, this spot disappears.

These data constitute evidence of a new recessive Mendelian mutation in the rabbit affecting the agouti pattern. That the responsible gene is not an allelomorph of the agouti series is shown by matings of an F_2 wide-banded male to non-agouti and to black-and-tan females, all of narrow-banded ancestry, from which 16 narrow-banded offspring have been produced.

It seems possible that the survival value of a gene of this nature under different environments may in part account for phenotypic differences in plains and mountain living forms of the rabbit. An animal bearing the wide band would be less conspicuous on a desert or on sandy plains than his darker prototype.

SUMMARY

1. Evidence is presented that dark, light and pale chinchilla are inherited as three different allelomorphs of albinism and of full color. Dark and pale were identified by Castle. Light is probably an allelomorph first recognized by Kosswig and Nachtsheim, but they were probably mistaken in thinking "Marder" distinct from "pale."

2. A comparative study has been made of the intensity of black pigmentation in various combinations of these allelomorphs, one with another and with full color, as well as with Himalayan albinism and complete albinism. The series is a graded one in which both coat pigmentation and pigmentation of the interior of the eye decrease simultaneously.

3. Blue eye, which is characteristic of dark chinchilla, is shown to be either one of the manifold effects of this allelomorph or due to mutation in a gene closely linked with it.

4. A variation in the intensity of pigmentation of the front surface of the iris is found to be due to two or more modifying genes inherited independently of the albino series. In dark chinchilla the range may be from a nearly clear blue through gray to pseudo-brown; in light and pale chinchilla, from light gray or translucent brown to dark brown; and in full color, from light gray brown to a very dark brown almost black.

5. Two genetic influences are therefore of primary importance in determining eye color in the rabbit. The first of these, the albino allelomorphs, determine an orderly and graded series of intensity in the coat and in the choroid and retinal epithelium of the eye and are responsible for the presence or absence of the red glow of the pupil by reflected light common to albinotic forms. They also determine a series of stages of decreasing iris pigmentation, parallel with that of coat color and retinal pigmentation, except in the case of the dark chinchilla allelomorph (ch^3) which has an iris lighter than those of the lower allelomorphs (ch^2 and ch^1) when no modifying genes interfere.

The second influence on eye color is that of independently inherited modifying genes affecting pigmentation only in the iris.

6. Three cases of heterochromia iridis are described which are probably to be explained as irregularities in the behavior of the genetic factors which modify the pigmentation at the front surface of the iris. A satisfactory explanation of these is dependent upon a more complete analysis of these factors.

7. Yellow which is scarcely able to come to expression in the chinchilla is manifested in "rusty back." This is a regional development of yellow in the baby pelage of dark chinchillas. It is inherited independently of the albino allelomorphs since it may be transmitted through pale chinchillas and albinos in which it is not manifest. It is possible that two factor pairs are involved which act in a cumulative manner. Rusty back is not found to be correlated with the modification of iris pigmentation, though inherited in much the same manner.

8. Certain analogies are indicated between the inheritance of blue eye in the chinchilla rabbit and in man.

9. A new variation is reported for the first time, consisting in an increase of the width of the agouti band on the secondary guard hairs and wool hairs. This behaves as a simple Mendelian recessive to normal band. It is not an allelomorph of the agouti gene.

ACKNOWLEDGMENTS

I wish to thank Dr. Hans Nachtsheim for supplying a Marder rabbit for comparison with the American Sable individuals, and Dr. Walter Landauer for bringing the Marder rabbit to this country. My thanks are also due to Professor I. W. Bailey for the preparation of the photographic plates, to Dr. A. B. Dawson for advice in histological technique, to Dr. Clyde E. Keeler for suggestions in regard to the preparation of the illustrations, and especially to Dr. W. E. Castle under whose supervision these experiments have been carried out.

LITERATURE CITED

- BOAS, H. M. 1919. Inheritance of eye color in man. *Amer. Jour. Physical Anthropology*, vol. 2, pp. 15-20.
- BOND, C. S. 1912. On heterochromia iridis in man and animals from the genetic point of view. *Jour. Genetics*, vol. 2, pp. 99-129.
- BROWNEE, J. 1913. Of inheritance of hair and eye color. *Proc. Roy. Soc. of Edinburgh*, vol. 23, pp. 458-474.
- BRYN, H. 1920. Researches into anthropological heredity. I: On the inheritance of eye color in man. *Hereditas*, vol. 1, pp. 186-198.
- CASTLE, W. E. 1905. Heredity of coat characters in guinea-pigs and rabbits. *Carnegie Inst. Wash. Pub. No. 23*, p. 78.
- . 1921. Genetics of the chinchilla rabbit. *Science*, n.s., vol. 53, pp. 387-388.
- . 1925. Heredity in rabbits and guinea-pigs. *Bibliographia Genetica*, vol. 1, pp. 419-458.
- . 1926. Studies of color inheritance and of linkage in rabbits. Part I. *Carnegie Inst. Wash. Pub. No. 337*, p. 47.
- . 1929. A mosaic (intense-dilute) coat pattern in the rabbit. *Jour. Exper. Zool.*, vol. 52, pp. 471-480.
- . 1929b. A further study of size inheritance in rabbits, with special reference to the existence of genes for size characters. *Jour. Exper. Zool.*, vol. 53, pp. 421-454.
- DAVENPORT, C. B. 1927. Heredity of human eye color. *Bibliographia Genetica*, vol. 3, pp. 443-463.
- DÜRKEN, B. 1925. Die Vererbung der Augenfarbe beim Meinschen. *Zeitsch. f. Indukt. Abstamm. u. Vererbungslehre*, Bd. 37, pp. 67-81.
- GATES, R. R. 1929. Heredity in man. *Macmillan Co.*
- GREGORY, P. W. 1929. Some new genetic types of eyes in the guinea-pig. *Jour. Exper. Zool.*, vol. 52 No. 1, pp. 131-153.
- GROSS, K. 1921. Über Vererbung von Augen und Haar Farbe und den Zusammenhang beider. *Arch. f. Rassen u. Gesellschaftsbiol.*, Bd. 13, S. 164-175.
- HOLMES, S. J., and H. M. Loomis. 1909. The heredity of eye color and hair color in man. *Biol. Bull.*, vol. 18, pp. 50-65.
- HURST, C. C. 1906. Mendelian characters in plants and animals. *Rep. Conf. Gen.*, Roy. Hort. Soc. London, p. 114.
- ILJIN, N. A. 1926. Studies in morphogenetics of animal pigmentation. II: Investigations of the temperature influence of the Himalayan rabbit's pigmentation. *Trans. Lab. Exper. Biol. Zoopark of Moscow*, vol. 1, pp. 11-63.
- KLEEFELD, HANS. 1929. Histologische Untersuchungen über die Elementarbestandteile des Kaninchenfelles unter besonderer Berücksichtigung der volkswirtschaftlichen Aufgaben der deutschen Kaninchenzucht. *Tierzucht und Tierhaltung*, Bd. 1, S. 1-51.
- KOSSWIG, C. 1927. Über die Vererbung und Bildung von Pigment bei Kaninchenrassen. *Zeit. f. indukt. Abstamm. u. Vererbungslehre*, Bd. 45, S. 368-401.
- LENZ, F. 1923. Die Akromelanie der Russenkaninchen und ihre Bedeutung für unsere Auffassung von der Akromegalie. *Arch. f. Rassen u. Gesellschaftsbiol.*, Bd. 15, S. 24-33.
- NACHTSHEIM, HANS. 1929. Die Entstehung der Kaninchenrassen im Lichte ihrer Genetik. *Zeit. f. Tierzucht und Zuchtsbiol.*, Bd. 14, S. 53-109.
- PAP, ANDRE. 1921. Über Vererbung von Farbe und Zeichnung bei den Kaninchen. *Zeit. f. indukt. Abstamm. u. Vererbungslehre*, Bd. 26, S. 185-270.
- PINCUS, G. 1929. A mosaic (black-brown) coat pattern in the mouse. *Jour. Exper. Zool.*, vol. 52, pp. 439-441.
- PUNNETT, R. C. 1912. Inheritance of coat color in rabbits. *Jour. Genetics*, vol. 2, pp. 221-238.
- , and M. S. PEASE. 1925. On the pattern of the Dutch rabbit. *Jour. Genetics*, vol. 15, pp. 373-406.
- RIDGWAY, R. 1912. Color standards and nomenclature. *Washington, D. C.* The author.
- SALAMAN, R. N. 1922. Inheritance of fur type and hair characters in rabbits. *Jour. Genetics*, vol. 12, pp. 179-208.
- SCHULTZ, WALTHER. 1918. Versteckte Erbfactoren der Albino für Färbung beim Russen kaninchen im Soma dargestellt und rein somatisch zur Wirkung gebracht. *Zeit. f. indukt. Abstamm. u. Vererbungslehre*, Bd. 20, S. 27-40.
- . 1922. Erzeugung des Winterschwarz. Willkürliche Schwärzung gelber Haare. *Arch. Entw. Mech. d. Organ*, Bd. 51, S. 338-382.
- . 1927. Willkürliche Augen pigmentierung bei Säugetier albinos. *Roux Arch.*, vol. 109, S. 287-301.
- . 1930. Luftmelaninbildung bei Zimmertemperatur in Säugetier augen und Haarwurzeln. *Arch. Entw. Mech. d. Organ.*, vol. 123, S. 132-152.
- . 1929. Pigmentierung von albino Augen und sympathetische Heterochromie der Iris Willkürlich erzeugbar. *Von Graefe's Arch. f. Ophthalmologie*, vol. 122, pp. 601-621.

- SNELL, GEORGE D. 1931. Inheritance in the house mouse, the linkage relations of short ear, hairless and naked. *Genetics*, vol. 16, pp. 42-74.
- STURTEVANT, A. H. 1913. The Himalayan rabbit case, with some considerations on multiple allelomorphs. *Amer. Nat.*, vol. 47, pp. 234-238.
- SUMNER, F. B. 1930. Genetic and distributional studies of three sub-species of *Peromyscus*. *Jour. Genetics*, vol. 23, No. 2, pp. 275-368.
- WINGE, O. 1921. On a partial sex-linked inheritance of eye color in man. *Compt Rend Lab. Carlsberg*, XIV. No. 12, pp. 23.
- WRIGHT, S. 1915. The albino series of allelomorphs in guinea-pigs. *Amer. Nat.*, vol. 49, pp. 140-148.
- . 1916. An intensive study of the inheritance of color and of other coat characters in guinea-pigs, with especial reference to graded variations. *Carnegie Inst. Wash. Pub. No. 241, Part II*, pp. 59-121.
- . 1918. Color inheritance in mammals: XI Man. *Jour. Heredity*, vol. 9, pp. 227-240.
- . 1923. Two new color factors of the guinea-pig. *Amer. Nat.*, vol. 57, pp. 42-51.
- . 1925. The factors of the albino series of guinea-pigs and their effects on black and yellow pigmentation. *Genetics*, vol. 10, pp. 223-259.

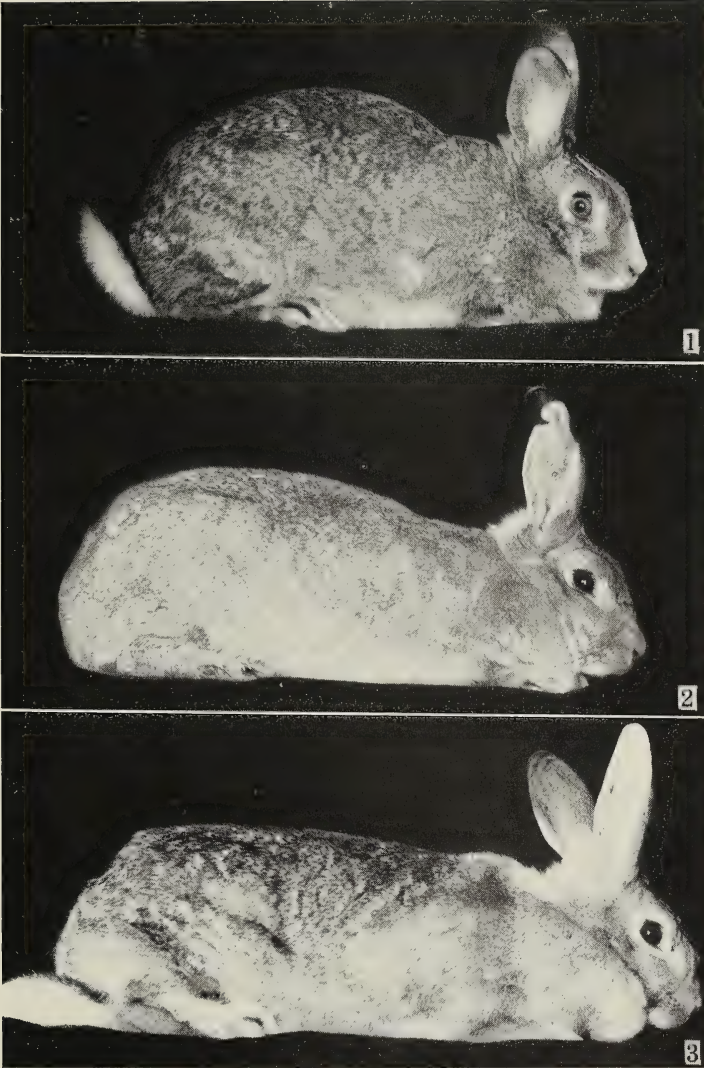


Fig. 1—Blue-eyed dark chinchilla (ch^3).

Fig. 2—Pale chinchilla. Note the less intense black on back and brown eye as compared with dark chinchilla, figure 1.

Fig. 3—Dark chinchilla (ch^3) with gray eye (grade 4 modified blue eye).

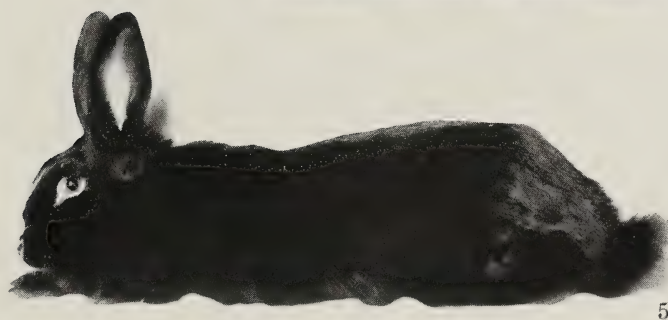


Fig. 4—Non-agouti dark chinchilla ($aach^3$) with a clear blue eye (grade 1). Note intense black pigmentation of fur. White hairs are a form of silvering due to genes not involved in this experiment.

Fig. 5—Non-agouti dark chinchilla ($aach^3$) with a clear blue eye (grade 1). Note intense black pigmentation of fur.

Fig. 6—Non-agouti light chinchilla heterozygous for albinism (ch^2c). Note darker eye and less intense black coat than in figure 5. Lighter spots on back and sides are due to old unshed fur and show tendency of coat to fade. This tendency is not found in dark chinchilla (ch^3).



Fig. 7—"Marder" rabbit from Germany presented by Dr. Hans Nachtsheim.

Fig. 8—Non-agouti pale chinchilla (or sable) heterozygous for albinism (ch^1c). Note intensely pigmented extremities (nose, ears, feet and tail) and close resemblance to "Marder" (fig. 7).

Fig. 9—Non-agouti individuals (age about 3 months) of three types of chinchilla. Note different intensity of pigment in coat of each. Individual at left is of genotype ch^3c , one in middle is a ch^2c genotype, and one at right is a ch^1c .

PLATE 4

FIG. 10—Eye of the White Dutch race of Castle. Note entire absence of pigment from front surface of iris.

FIG. 11—Eye of the Vienna White race of Castle. Pigment is entirely absent from front surface of iris, as in White Dutch (fig. 10).

FIG. 12—Grade 1 blue eye of a dark chinchilla (ch^3). Small amount of pigment is present in small flecks in front surface of iris.

FIG. 13—Grade 2 modified blue eye of dark chinchilla (ch^3) or a gray eye.

FIG. 14—Grade 3 modified blue eye of dark chinchilla (ch^3), also a gray eye, but with a greater amount of pigment.

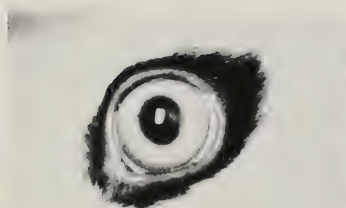
FIG. 15—Grade 4 modified blue eye of dark chinchilla (ch^3).

FIG. 16—Light gray or translucent brown eye of light chinchilla (ch^2) comparable genetically to grade 1 blue eye of dark chinchilla.

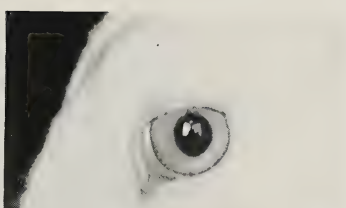
FIG. 17—Dark brown eye of light chinchilla (ch^2) genetically similar to grade 4 or 5 blue eye of dark chinchilla.

FIG. 18—Light gray-brown eye of full-colored rabbit comparable with grade 1 blue eye of dark chinchilla.

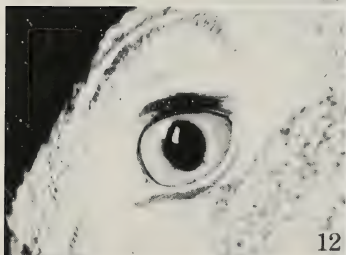
FIG. 19—Dark brown eye of full-colored rabbit genetically similar to grade 4 or 5 blue eye of dark chinchilla.



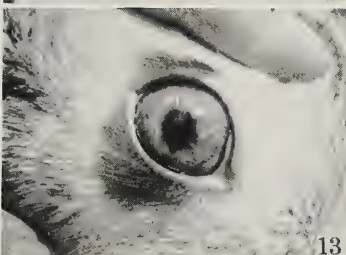
10



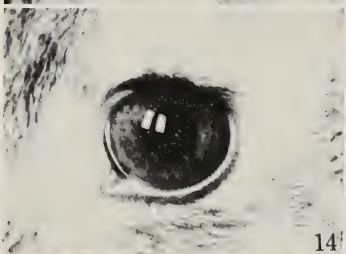
11



12



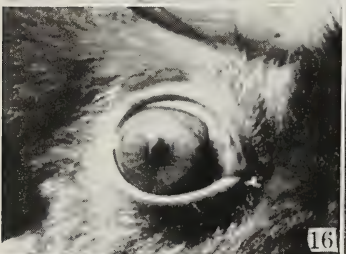
13



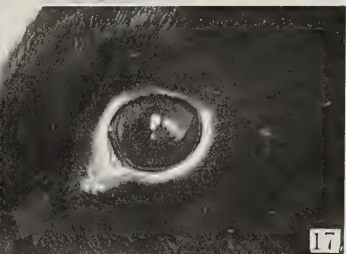
14



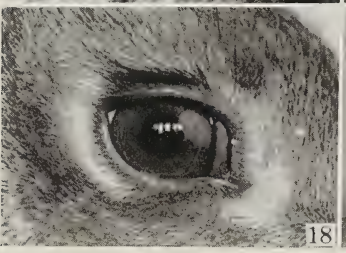
15



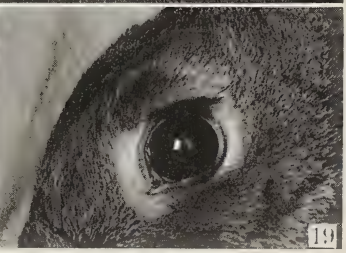
16



17



18



19

PLATE 5

FIGS. 20 to 25—Sections of iris of various albino allelomorphs, arranged in each case with anterior surface of iris at top. Microphotographs.

FIG. 20—Full color, dark brown eye, with maximum amount of pigment on front surface of iris. Retinal epithelium is intensely packed with dark pigment granules. Numerous highly branched chromatophores are scattered throughout iris stroma and especially around blood-vessels and at front surface of iris.

FIG. 21—Full color, light brown eye with maximum amount of pigment on front surface of iris. Retinal epithelium of iris is also thickly packed with dark pigment granules. Fewer branched chromatophores are found at front surface and stroma is comparatively free from pigment.

FIG. 22—Pseudo-brown eye of dark chinchilla (ch^3), with maximum amount of pigment on front surface of iris. Pigmentation of retinal epithelium is slightly reduced as indicated by its interrupted character. Stroma is comparatively free from pigment except some highly branched chromatophores at surface of iris. Note tendency of chromatophores to clump, with unpigmented areas between.

FIG. 23—Grade 2 modified blue eye of dark chinchilla (ch^3), with a minimum of anterior iris pigmentation. Clumping of chromatophores is clearly shown with much larger unpigmented areas between, resulting in blue of lower grades of blue eye in living animal.

FIG. 24—Light brown-eyed pale chinchilla. A maximal amount of pigment is seen at front surface of iris. Note tendency for processes of chromatophores to run at right angles to surface of iris. A greater tendency is also shown for production of pigment in branched chromatophores throughout stroma though much more diffusely than in full color. Pigmentation of retinal epithelium is greatly reduced.

FIG. 25—Very light brown eye of pale chinchilla with a minimal pigmentation throughout entire iris. Front surface has practically no pigment, and retinal epithelium is small in amount.

Abbreviations:

- A, anterior surface of iris
- S, stroma
- R E, retinal epithelium
- C, branched chromatophores
- B V, blood-vessel

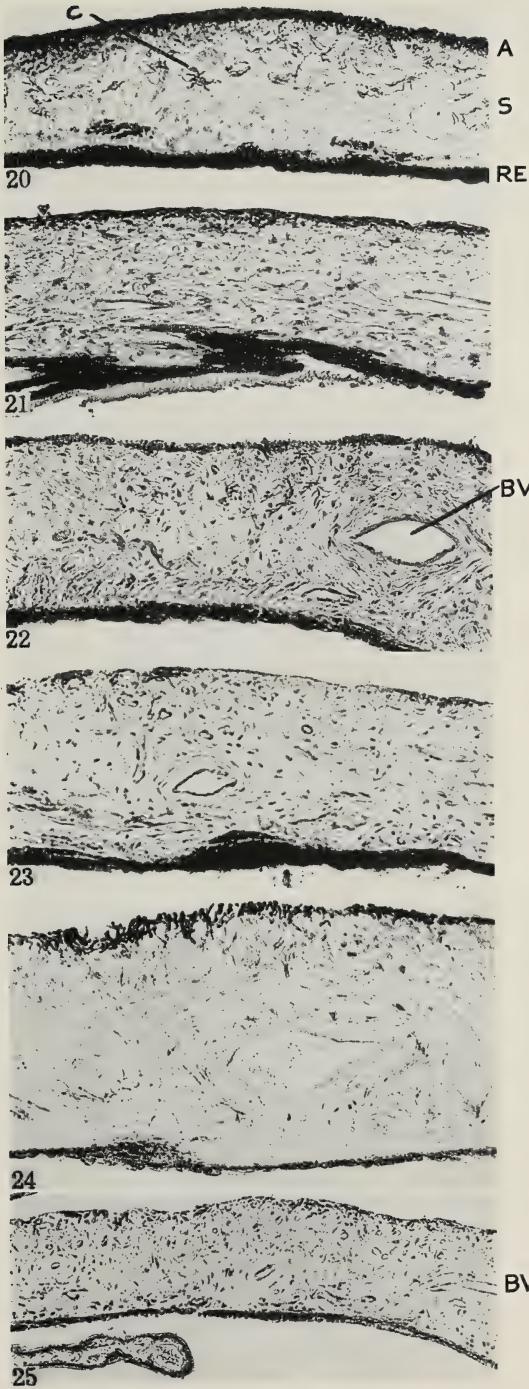


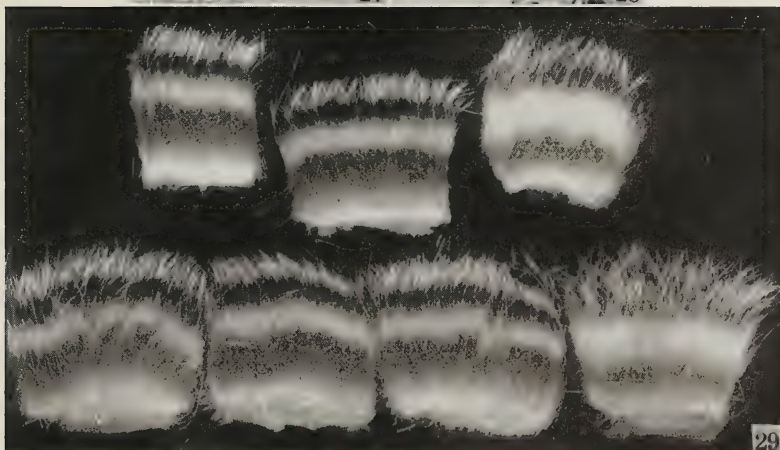
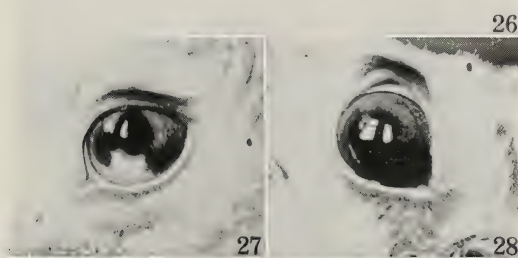
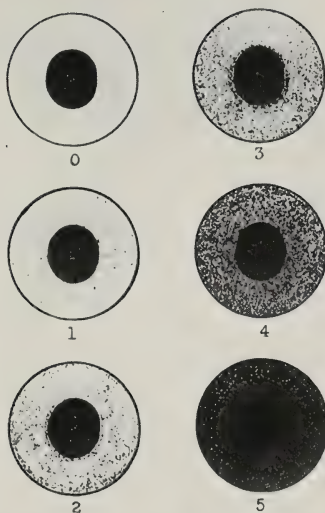
PLATE 6

FIG. 26—A scale of grades of intensity of pigment seen on front wall of iris surrounding pupil. Grade 0 shows total absence of pigment as in eye of "White Dutch" (Castle, 1926) and Vienna White. Grade 1 is condition found in clearest blue eyes of dark chinchilla (ch^3) individuals. Grade 5 is "pseudo-brown," most extreme modification of blue eye, and grades 2, 3 and 4 are intermediate types.

FIG. 27—Heterochromic iris of Dutch marked dark chinchilla female 14A1. Ventral sector is blue of White Dutch. Dorsal sector is dark chinchilla blue eye of grade 3 and lateral sectors are pseudo-brown.

FIG. 28—Heterochromic eye of dark chinchilla (ch^3) female 27R1. Ventral sector is pseudo-brown, dorsal sector is of a grade 3 modified blue pigmentation.

FIG. 29—Hair samples from individuals concerned in cross between narrow-banded and wide-banded chinchilla rabbits; P1, narrow-banded at left, wide-banded at right; F1, all narrow-banded; F2, a 3:1 ratio of narrow-banded to wide-banded.



P₁

F₁

F₂

29

SMITHSONIAN INSTITUTION LIBRARIES



3 9088 00153550 9

nhmamm QH431.C263X

Contributions to the genetics of the dom